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PHYTOPATHOLOGY

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MARCH, 1935

DISSEMINATION OF SOUTHERN CELERY-MOSAIC VIRUS ON VEGETABLE CROPS IN FLORIDA

F. L. WELLMAN

(Accepted for publication August 17, 1934)

INTRODUCTION

The southern celery-mosaic virus (*Celery virus 1*) has been described by the writer (12). In field studies, reported in the present paper, of diseases caused by this virus, it was evident that a characteristic pattern of spread of the disease occurred in Golden Self Blanching and other varieties of celery, in Cocozelle and Golden Summer Crookneck varieties of squash, and in peppers of the California Wonder and World Beater varieties. There was some difference in disease spread in maize (sweet corn). The type of disease spread as it was noted in celery, peppers, and squash was unchanged in its attack on beds of 2 weed hosts, *Commelina nudiflora* L., and *Ambrosia elatior* (*artemisiifolia*) L.

In consulting the voluminous plant-virus literature one finds unanimity of opinion and numerous references to the fact that these diseases spread, but there has been but little detailed discussion of this phase of the problem. Allard (1) described how a small colony of aphids brought mosaic into a tobacco seed bed at the point of establishment of the colony. When transplanted from this seed bed sporadic cases of the disease occurred and, with increase in spread of insects, the disease was scattered throughout the field until nearly all plants were affected. Goss (8) stated that increase in aphid populations probably accounted for increase in potato-viroses. Gardner and Kendrick (7) found that tomato mosaic overwintered in wild hosts and could spread 40 rods. Doolittle and Walker (4) found that cucumber mosaic spread to cucumbers from diseased wild hosts in the field or along the edges. Folsom, Schultz, and Bonde (6) discovered in their work that dissemination of certain potato viroses extended across rows and from field to field. There was also a correlation between proximity and mosaic increase in the first rows next to diseased stock, and dispersal appeared to be accounted for by flight of infective insects. Smith (10) summarizes literature on virus dissemination and includes a list of citations (q.v.). It is notable that there have been no major detailed reports of field dissemination, pattern, or progress of spread.

MATERIALS AND METHODS

In the greenhouse studies the same strain of the virus was used as that employed in the writer's identification studies (12) of *Celery virus 1*. In all cases of apparent spread of the virus in the field, representative affected plants of different species from diseased fields were collected, the virus was recovered, and its identity determined by comparison with the known *Celery virus 1*. Aphids used in greenhouse studies were descendants of known cultures of *Aphis gossypii* Glov. maintained by S. P. Doolittle at Washington, D. C. Virus-free colonies were kept in insect-proof cages on healthy plants. Seeds of vegetable crops used in these studies were of named varieties obtained from reputable dealers, and specific identification of weeds were made by S. F. Blake of the Bureau of Plant Industry.

The field work reported in this paper was accomplished during the winters of 1930–31 to 1933–34, in Florida. General observations were made repeatedly in several parts of the State, and careful counts, maps, and detailed studies were made in the district around Sanford, Fla.

PATTERN OF DISTRIBUTION OF DISEASED PLANTS IN THE FIELD[1]

Diseases caused by the southern celery-mosaic virus spread in a generally characteristic pattern in fields of various varieties of squash, pepper, and celery. Initial appearance usually occurs on the edge of the field where there may be an infection source. This may be diseased crop plants of the same species or of other species or it may be a fringe of diseased wild host plants. After introduction into the field the disease spreads inward but it does not necessarily extend to make a fan shaped pattern, nor is there a frontier border of spread progressing in an unbroken line as a wave would advance. Dissemination is evident principally as a successive development of small scattered areas which, upon expansion, coalesce and in turn result in further infection and widely dispersed centers of disease. This may continue until the whole field is completely involved.

Spread of the celery virosis on celery, *Apium graveolens* L., was studied with particular care during 4 winters in sections of a number of fields in the Sanford district. One such plot (Fig. 1), studied in the winter of 1932–33, is a fairly typical example of the spread of this disease. This plot was planted in November with 1050 celery plants from a well sprayed virosis-free seed bed. In this plot there were 14 rows, 30 inches apart, with plants, all of the Golden Self Blanching variety, 3½ inches apart in the row. Two small patches of weeds, composed largely of *Commelina nudiflora* and *Geranium carolinianum* L. were located along the eastern edge of the plot. Both species were diseased by a virus that proved to be *Celery*

[1] The diagrams in this section were prepared by F. S. Beecher, Bureau of Plant Industry, from the writer's field diagrams.

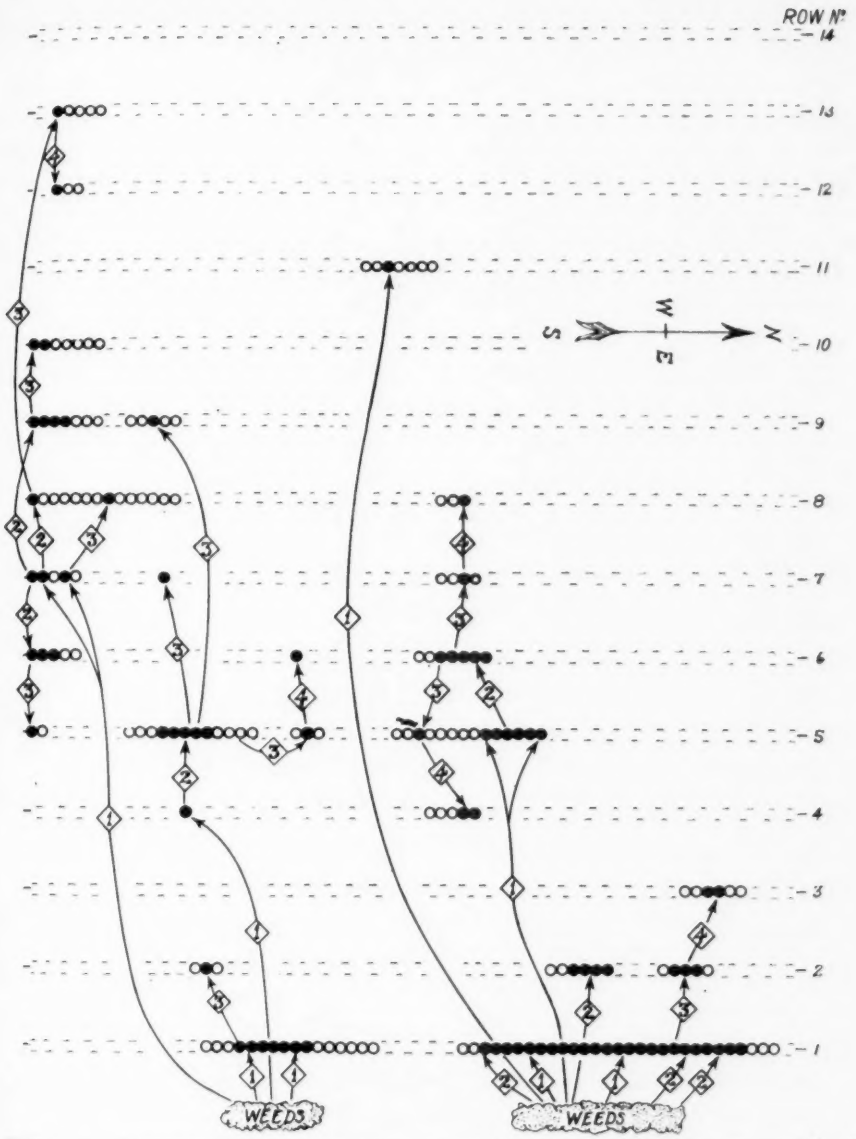


FIG. 1. Diagrammatic representation of results from field studies of spread of celery virus in Golden Self Blanching celery plants. Primary diseased plants are represented by black spots, circles are secondary diseased plants adjacent to primary infected individuals, and arrows represent probable course of travel of viruliferous aphids to new areas of infection. Arrows numbered 1 lead from weeds acting as original sources of infection to areas of plants that first appeared as diseased on December 1. Arrows numbered 2 lead from old to new areas of infection noted December 11. Aphids disappeared from weeds at this time but multiplied rapidly on celery. Arrows numbered 3 lead from previously infected areas to plants found diseased on December 24. Arrows numbered 4 represent course of spread to new diseased areas observed January 12. On January 20 there were few aphids and no new diseased areas. Newly infected plants all occurred in rows next to plants already diseased. Generally speaking, spread of the disease was in all directions, though greatest length of spread was from east to west, the direction of the warm east trade winds prevalent during the period of these observations. (For successive increases in numbers of diseased plants, see text and the figures given for plot I in table 1.)

virus 1, and were liberally infested with *Aphis gossypii*, which were multiplying upon them at the time the plot was planted. These aphids left the weed hosts and moved to the celery shortly after it was put into the field. This migration is common and is not necessarily induced by planting and cultivation of fields adjacent to weed patches. Wingless aphids were found to crawl away from plants colonized by aphids, at almost any time and in any direction, in search of new pasturage. Winged individuals were observed to leave their parent colonies early in the morning. They flew toward the sun but were usually carried back on the warm and light easterly breezes then prevalent, alighting at considerable distances west of the plants from which they originally flew.

On November 29 the plot had been planted 15 days. It was carefully examined and some plants with suspicious markings were noted but none could be found with definite symptoms of virus infection. Diseased weeds on the edge of the field had several aphids on them, and 40 celery plants in the field were found infested with 1 to 7 winged and wingless aphids. On December 1, aphids were found in about the same numbers on celery and weeds, and there were 6 areas in the field in which a total of 26 virus-diseased celery plants were found. These plants all seemed to have been originally inoculated by viruliferous aphids coming from the weed patches, as indicated on the diagram (Fig. 1) by arrows numbered 1. One hundred ninety-two plants carried aphids at this time, and it was evident, since only 26 of these were diseased, that not every plant that harbored an aphid had succumbed to virus infection.

On December 11, no aphids were found on the weeds at the edge of the plot. Aphids were, however, becoming more numerous on the originally infested celery plants, were spreading to surrounding individuals. Six new infection areas were noted (Fig. 1) that resulted apparently from aphid migration both from weed patches and from the first areas of plants infected. Thirty-seven new diseased celery plants, making a total of 62 plants with mosaic symptoms, were recorded, the apparent source of new infections traced and indicated by arrows marked 2. Aphids were not abundant anywhere in the plot on December 11. By December 24, further multiplication and spread of viruliferous aphids had occurred. No aphids could be found on the weeds, but 11 new virus-infected areas of celery plants had appeared with 12 newly infected plants, all of which (Fig. 1) seemed to have been initiated by aphids from originally infected plants noted December 11. The course of this spread was designated by arrows marked 3 (Fig. 1). On December 24 aphids had become more plentiful in this field, and the first secondary spread of the disease in plants next to the primary infected individuals in the row was definitely observed. Twenty such individuals were found and are denoted as circles in the diagram (Fig. 1).

The plot was not visited again for over 2 weeks and on January 12 further spread had taken place and the course of spread into the 7 new areas (Fig. 1) was traced. This is signified by arrows marked 4 on the diagram. It appears that in every case this extension originated in primary infected celery plants that had been previously diseased. A marked diminution in aphid population also was noted. At the same time, 33 celery plants, part of those designated by circles in figure 1, had succumbed to the disease and evidently had been inoculated by insects from diseased plants growing next to them in the same row. The next observations were made January 20, when aphids had all but disappeared and no new areas of infection appeared in the field. There were, however, 37 additional plants infected through contracting the malady from other individuals next to them in the same row. At that time a total of 175 plants were diseased.

It was not possible, because of pressure of other field work, to make regular subsequent counts of diseased plants. General observations, however, were made. Very few aphids could be found and almost no increase in disease occurred until late February, when a severe infestation of aphids came in from infested, though virus-free, squash in a near-by field. This adjoining field of squash had recently been chopped down preparatory to replanting. Upon harvesting the plot of celery, March 24, the weeds were found to be free from aphids, though large numbers of the insects were present on the celery. There were 928 diseased plants in the plot, which was 88 per cent of the population. A week after harvest the weeds at the edge of the plot were again found to be well infested with numbers of *Aphis gossypii*.

A more general view of the pattern and course of spread of the celery virus in a celery field was obtained in the winter of 1930–31. Along one edge of this field, diagramatically represented in figure 2, A, B, C, and D, there were 7 patches of weeds all having severe symptoms of virus attack. The weed species consisted of *Commelina nudiflora*, *Geranium carolinianum*, *Ambrosia elatior* (*artemisiifolia*), *Bidens leucantha* (L.) Willd., and *Chenopodium botrys* L. Subsequently, by inoculation studies, all these weeds were proved to be infected with *Celery virus 1* with the exception of Bidens and Chenopodium.

The disease appeared in the field first along with a scattering of aphids, *Aphis gossypii*, in a few plants close to virus-infected weed patches. By December 24, a total of 16 diseased plants were located and the areas in which they were found were noted (Fig. 2, A). The second date of observing virus-disease progress in this field was January 20, 27 days later than the first (Fig. 2, B). There were 110 diseased plants and the spread extended westward and was distinctly correlated with the location of beds of diseased weeds. Rapid and concentrated invasion was particularly notable

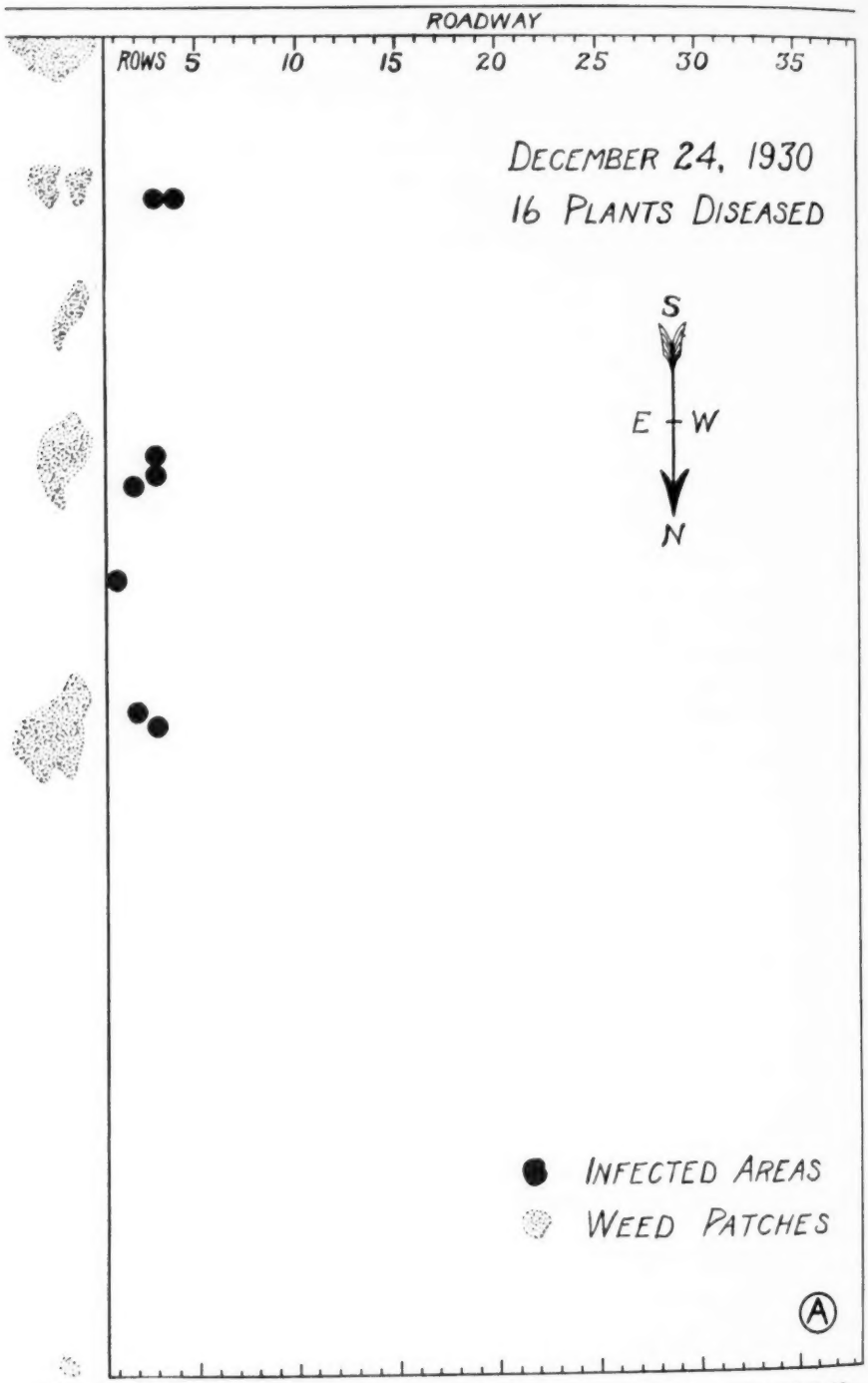
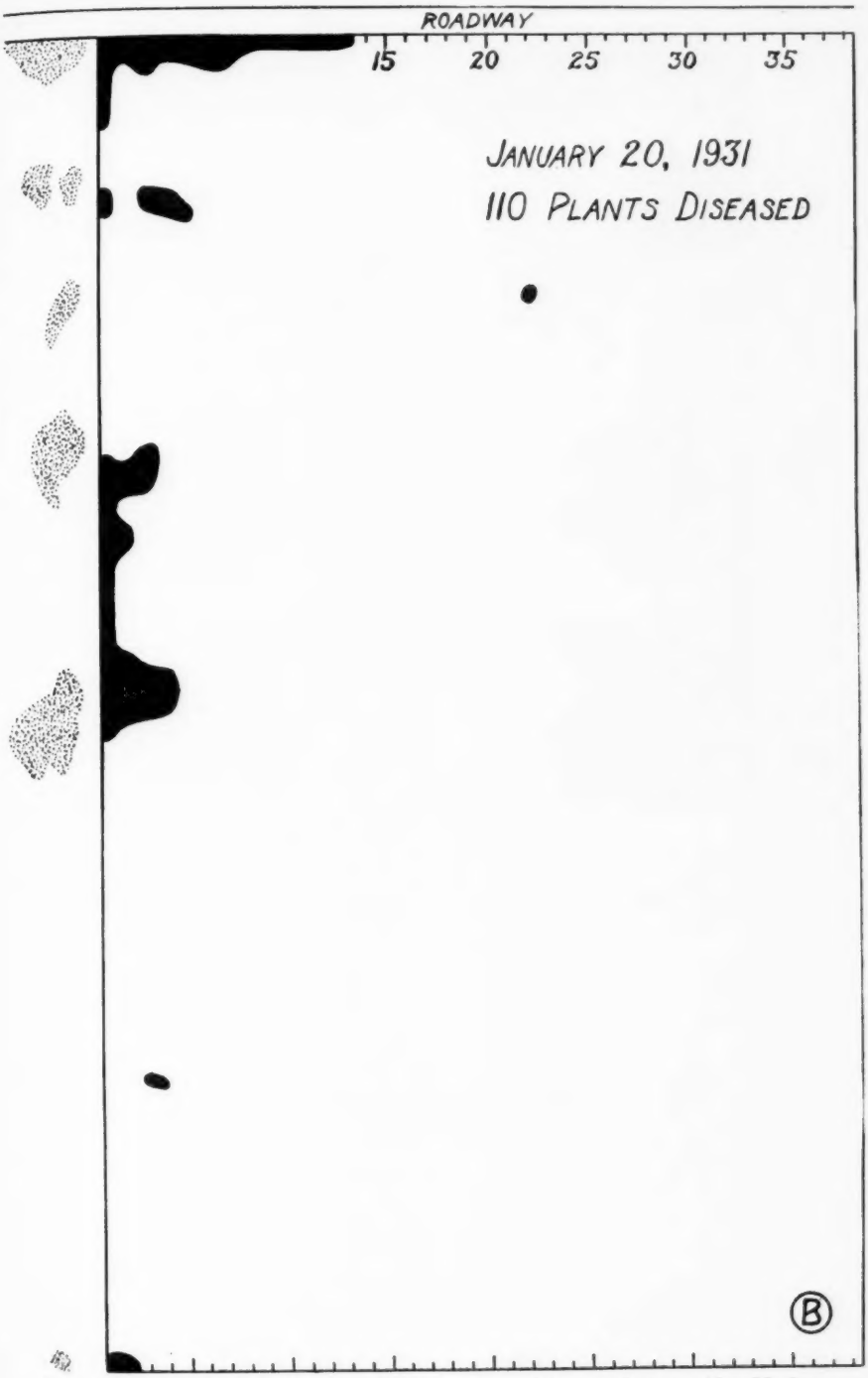
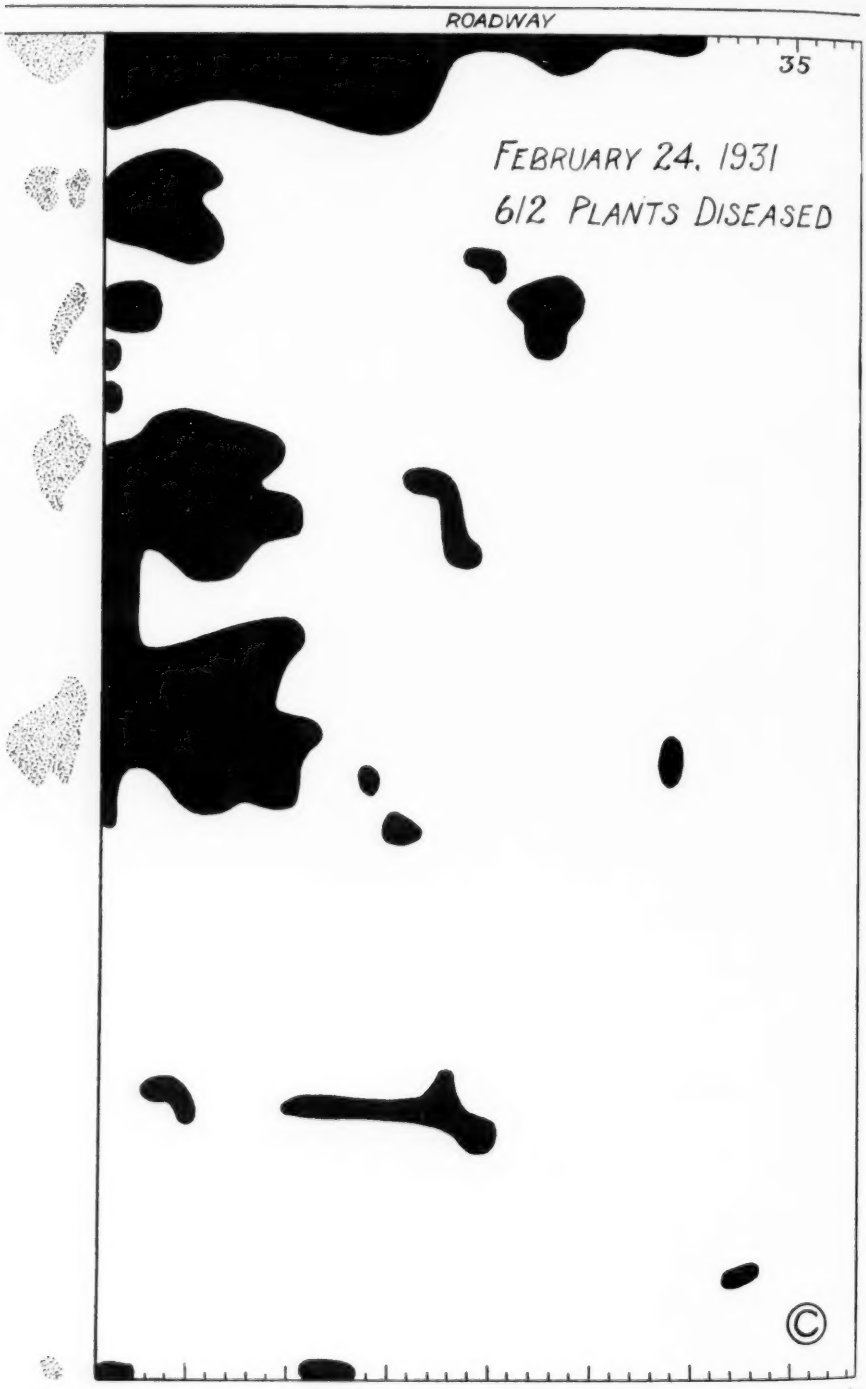


FIG. 2. Diagrams showing successive spread of the celery virus in a field of Golden Self Blanching celery, near Sanford, Florida.

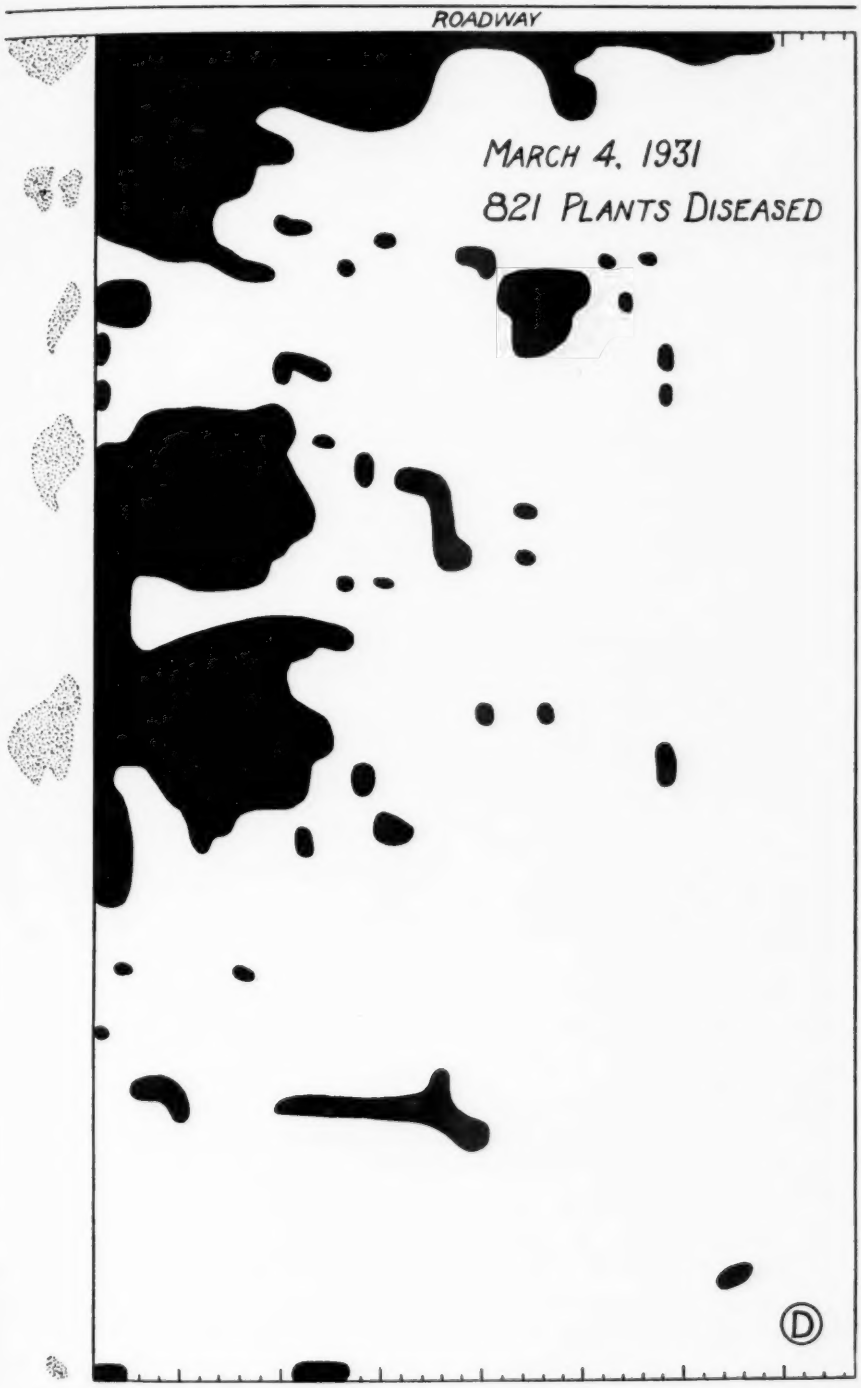
A. Note that all diseased plants to be found at the beginning of observations were in the first 4 rows, and were definitely correlated with position of weed patches at eastern edge of field.



B. Spread had progressed rather slowly in a month's time but considerable increase in numbers of diseased individuals was, nevertheless, noticeable. There were also 2 well-isolated areas where disease appeared, one of them being about 45 feet from the nearest infected plants.



C. It was found on this date that spread had progressed very rapidly in the ends of the rows along the weed-free roadway. Also, that general expansion of infected areas occurred at the time of appearance of numerous isolated areas of infection.



D. The last observations were made somewhat over a week later. The total number of diseased plants had increased greatly, as well as the numbers of isolated areas of disease.

along the weed-free roadway on one side of the field. The ends of the first 13 rows of plants abutting upon this roadway were especially affected. The third observations were made 35 days later, on February 24. At this time, 612 plants showed virus attack (Fig. 2, C), spread had advanced further into the field, and at considerable distances there were scattered local areas of infection completely surrounded by healthy plants. The disease appeared to be spreading more rapidly in a westward than in an eastward direction. The last counts and observations were made on March 4 (see Fig. 2, D). In a period of 8 days the total of diseased plants had increased to 821, the general regions of infection had become considerably extended, and numerous small assemblages of mosaic plants had become established around the outside edges of the areas of spread, though the smallest infected areas are not indicated in figure 2, D. This celery was harvested nearly 4 weeks after the writer made his last counts. The owner of this field reported that the disease had spread in severe form during the remainder of the month of March, to considerably more than twice the area affected on March 4, and that all sections of the plot were supplied with numbers of small areas of diseased plants. Severe losses are sustained in such a field at harvest time, as is illustrated in figure 3.

In studying the pattern of spread of celery virus in squash and pepper fields it was found to be of the same type as that described for the trouble in celery. It differed especially in squash fields in that infected areas were

FIG. 3. Photograph toward center of a celery field at harvest time showing the large majority of celery plants that were infected with southern celery mosaic so disfigured that they were cut down and left in the field because of unsuitability for shipment. (Photograph by S. P. Doolittle.)

FIG. 4. Photograph of month-old 10-acre field of Golden Summer Crookneck squash severely diseased with *Celery virus 1* originating from infected weeds (*Commelina nudiflora*, *Ambrosia elatior*, and *Geranium carolinianum*) growing in the shallow ditch seen in the foreground. This field had a perfect stand of squash seedlings at the time the virus started to spread. Aphids migrated from the weeds, infected the squash plants, multiplied, and migrated rapidly to all parts of the field. Note especially dead and dying plants in the bare area over an extensive portion of the field, with a few rows of more mildly affected squash plants in the distant background. Field corn was being planted between the squash rows at the time this picture was taken, and sometime later the squash plants were all hoed out, no salable crop having developed.

more conspicuous and more rapidly involved (Fig. 4). In fields of the sweet varieties of maize, spread was more or less confined to plants near the field edge, where the original source of infection occurred. Isolated diseased areas were found occurring well into the field, but they did not serve as infection centers from which the disease spread greatly, as in fields of celery, squash, and peppers. The accompanying illustration (Fig. 5) shows a maize field in which the disease is mostly confined to the first row next to celery-virus-diseased weeds, Ambrosia and Commelina, in a roadside ditch. In this field nearly every plant in the first row was infected and the disease was prevalent in the first 5 rows. While infected plants were found 25 rows away from this edge, the majority of the infected plants occurred close to the field edges.

In studying the spread of the virus from plant to plant in growths of the weed, *Commelina nudiflora*, the pattern of spread appeared similar to the typical form described as occurring in celery fields. In January, 1933,

FIG. 5. The spread of *Celery virus 1* in a field of maize (sweet corn) by aphids (*Aphis gossypii*) that had fed on infected weeds (*Ambrosia elatior* and *Commelina nudiflora*) growing at the edge of the field. Note stunting of diseased corn plants in first row. Nearly every plant in this row was affected, the disease was most prevalent in the first 5 rows, but could be found with ease 25 rows from the edge.

a 4-acre field, which had been uncultivated for a season, was found covered under the sparse growth of a leguminous cover crop, by an almost complete mat of *C. nudiflora*. This mat, apparently the result of germinated seeds, was free from virus infection with the exception of a few small diseased areas occurring toward the middle of the field, and a fringe of infected plants along the edge where there were some diseased *Geranium carolinianum* and the remains of an old field of celery-virus-infected peppers. In a month's time the fringe of infection had advanced into the field and become amalgamated with the isolated spots. The front line of infection had extended about 30 feet in from the edge of the field where the infection originated. Spread of the disease had almost ceased by the last of March, though it proceeded during the summer, and by the first week of September nearly all Commelina plants in the field were diseased. Several abandoned fields where the ragweed, *Ambrosia elatior*, had been allowed to grow were watched for spread of the disease. The pattern of spread of the celery virosis in these patches of weeds was essentially the same as that described for celery.

INCREASE IN NUMBERS OF DISEASED PLANTS IN CELERY FIELDS

Observations were undertaken in celery fields to study increase of disease in plots and the number of diseased plants in isolated areas where infection originated from single diseased individuals.

Successive counts of virus-diseased plants were secured, and the data have been assembled in tables 1, 2, and 3. Close study has shown that consecutive increases in the number of infected plants in fields are dependent on: the sources of infection, proximity of the sources to the crop under investigation, temperature and humidity relations, multiplication and migratory movements of the insect vector, size and shape of the plots, and methods of culture employed in the crops affected. Work on these factors is not complete and no attempt has been made here to correlate the data with increases of the disease.

TABLE 1.—*Increases in numbers of celery-virus-diseased plants at intervals during one season in plots of Golden Self Blanching celery*

Plot	Plants in plot	Observation	Plants diseased	Origin of virus	Presence of aphids
A	41,838	Dec. 24, 1930	16	Weeds	Weeds and celery
		Jan. 20, 1931	110		Celery only
		Feb. 24, "	612		" "
		Mar. 4, "	821		" "
I	1,050	Nov. 29, 1932	?	Weeds	Weeds and celery
		Dec. 1, "	27		Celery only
		" 11, "	62		" "
		" 24, "	94		" "
		Jan. 12, 1933	134		" "
		" 20, "	175		" "
		Mar. 24, "	928		" "
L	1,000	Nov. 6, 1933	1	Weeds and celery	Celery
		" 11, "	5		"
		" 21, "	15		"
		Dec. 1, "	41		"
		" 12, "	88		"
		Jan. 1, 1934	169		"
		" 12, "	288		"
		" 24, "	380		"
		Feb. 17, "	552		"
		" 27, "	736 (at harvest)		"
		Mar. 6, "	.....		"
N	1,000	Nov. 6, 1933	1	Celery	None
		" 11, "	1		"
		" 21, "	1		"
		Dec. 1, "	1		"
		" 12, "	1		"
		Jan. 1, 1934	2		Celery
		" 12, "	6		"
		" 24, "	16		"
		Feb. 17, "	37		"
		" 27, "	42 (at harvest)		"
		Mar. 6, "	.....		Weeds

The data presented in table 1, indicate the successive increases of diseased plants that occurred in plots. The mere occurrence of diseased plants in fields does not imply disease spread. Diseased plants may long be present in fields before spread of infection begins. The writer has known many instances of field infection derived from isolated diseased plants obtained from seed beds containing a few infected seedlings at planting time. In 4 such fields aphids had been destroyed in the seed bed and did not become abundant later in the season, diseased plants escaped infestation, and the virus was not spread to other plants during the whole course of the season. In plot N (Table 1) a single diseased plant was located in the center of a 1000-plant plot, and did not become a source of disease spread for 2 months, when aphids had colonized on it and then proceeded to spread the virus. In plots A, I, and L, in which spread occurred from the first, infection originated from weeds at the edge of the field and aphids were always present.

In the 1930–31 season, which was unfavorable to the spread of southern celery mosaic, observations were made with regard to spread of disease around 20 single newly infected individuals in marked locations in the fields (Table 2). These studies were made on 2 adjacent farms, and the isolated

TABLE 2.—*Spread of southern celery-mosaic virus to other individuals about single, originally diseased Golden Self Blanching celery plants in the field (Winter 1930–31)*

Original plant	Date diseased	Spread in original row		Spread to adjacent rows[b]		Spread at close of season	
		No. plants	Farthest spread[a]	No. plants	Occurrence	Total plants	Days required
a	Dec. 9	2	4 inches	0	.....	2	87
b	" 11	5	12 "	0	.....	5	85
c	" 11	13	36 "	32	both sides	45	84
d	" 11	5	12 "	3	one side	8	85
e	" 20	4	12 "	5	both sides	9	76
f	" 20	13	28 "	39	" "	52	74
g	Jan. 1	6	20 "	1	one side	7	65
h	" 1	2	8 "	0	.....	2	63
i	" 1	4	8 "	3	one side	7	65
j	" 1	5	8 "	4	both sides	9	64
k	Dec. 24	4	16 "	0	.....	4	81
l	" 24	7	16 "	10	one side	17	80
m	" 24	5	8 "	0	.....	5	79
n	Jan. 1	2	4 "	1	one side	3	64
o	" 1	4	8 "	2	" "	6	63
p	" 1	5	12 "	12	both sides	17	65
q	" 1	2	4 "	5	" "	7	65
r	" 1	14	52 "	27	" "	41	65
s	" 1	3	8 "	9	" "	12	65
t	Dec. 7	5	16 "	1	one side	6	89

[a] Plants were set 4 inches apart in the row.

[b] Rows were 30 inches apart in the field.

locations chosen were widely scattered, between 75 and 150 feet away from patches of virus-infected weeds or field edges. It is of considerable interest that so much variability occurred in the amount of spread of mosaic from an originally diseased plant to those surrounding it. In 2 cases (Table 2, plants a and h) the disease spread to only one other plant next a diseased individual, while within a few rods of those plants the disease spread to 51 plants from a single primary infected individual (Table 2, plant f). Of equal interest is the fact that in 1930–31, a season in which there was but little celery mosaic spread, as many as 41, 45, and 52 plants became diseased in areas in the field all started from single infected individuals. Similar studies were again attempted in 1931–32 and 1932–33. These seasons were warm, and in all cases data were so complicated with vigorous scattering spread of the disease that counts were not completed. In these seasons the disease spread from individual plants would have been considerably greater than any reported in table 2.

One method of expressing the importance of a disease in the development of the host is to determine the total number of plants in a certain population affected with the disease. In studying the virus of southern celery mosaic, counts were made in field plots at the time of year when the disease was first making its appearance. Later on, another count was made to determine in some measure how much the disease had increased. This increase of diseased plants (Table 3) was obtained in 14 different plots of celery during 4 seasons of observations. In addition, general con-

TABLE 3.—*Numbers of plants infected with Celery virus 1 at beginning and close of season of observation on field plots of Golden Self Blanching celery*

Plot	Total plants in plot	Counts of diseased plants		Date of first count	Days to last count
		First count	Last count		
A	41,838	16	821	12/24/30	67
B	39,600	66	34,542	12/11/30	83
C	29,896	36	3,348	12/11/30	86
D	4,200	14	43	12/24/31	22
E	4,200	67	308	12/24/31	22
F	5,418	126	1,592	12/1/31	44
G	5,160	138	1,508	12/24/31	22
H	14,133	8	89	12/24/31	22
I	13,608	7	76	12/1/32	46
J	5,418	92	1,572	12/1/32	46
K	1,050	26	928	12/1/32	115
L	1,000	1	736	12/21/33	113
M	1,000	1	6	11/21/33	83
N	1,000	1	42	12/12/33	113

firmatory observations were made on a large number of fields in the neighborhood of the plots. It is not exceptional for such large proportions of plants to become diseased as are, for example, indicated in plots B, C, F, G, J, and K, and this furnishes further verification of the fact that the virus, unless curbed in its attacks, might well become a limiting factor in the crops produced from certain cultivated host plants. It should be noted in passing that the virus has a very wide host range, subsequently to be reported upon, and that it is especially severe in plantings of pepper and squash.

FACTORS INFLUENCING FIELD DISSEMINATION

The primary occurrence of the celery virus in cultivated crops depends on the presence of adjacent diseased plants affected with the celery virus, and on the agency of spread. Methods of spread without aphids have been studied in the field, and experimentally under insect-free conditions. In a minor way spread of the virus results, in pepper and squash fields, from bruising incurred in the handling of plants in the hoeing process, and in the harvesting of fruits, while in celery it may take place by the continued bruising and exceedingly close contact of these plants as they stand in the row after they are about half-grown. Spread from contacts of celery plants in the row, however, may be practically disregarded as a factor of much economic importance in the dissemination of celery virosis.

The work of Doolittle and Wellman (5) proved that *Celery virus 1* could be transmitted by *Aphis gossypii* from *Commelina nudiflora* to celery, and *vice versa*, that the aphid might also transfer the virus from *Physalis lagascae* R. & S. to celery, and that the insect could successfully inoculate cucumber, tomato, *Physalis pubescens* L., and tobacco. The writer has recently reported (13) that *Aphis gossypii* carried the celery virus to certain of the Gramineae: *Zea mays* L., *Euchlaena mexicana* Schrad., *Holcus sorghum* L., and *Secale cereale* L. The writer also reported (14) that *Aphis gossypii* and *A. maidis* Fitch carried the virus to 2 species of banana, *Musa cavendishii* Lamb and *M. sapientum* L. Other aphid species are being studied as vectors of *Celery virus 1*.

The writer has pointed out (11) that *Aphis gossypii* is the important insect vector of the celery virus on celery around Sanford, Florida, and in that district it is called the celery aphid. The question arose as to whether *A. gossypii* might also act as the vector of *Celery virus 1* in a number of plant species not heretofore studied under controlled conditions but to which the virus was known to spread in the field. From 50 to 80 plants of every variety and species used, making up 5 to 8 series in each case, were studied in the greenhouse. It was found that *A. gossypii* was a potent vector of celery virus in the following varieties and species of plants: *Cucurbita pepo* L. var. *condensa* (Cocozelle and Golden Summer Crookneck

squashes), *Capsicum annuum* L. var. *grossum* (California Wonder and World Beater peppers), *Ambrosia elatior*, and *Geranium carolinianum*. Photographs of pepper and squash plants diseased with the celery virus upon inoculation with viruliferous *Aphis gossypii* in the greenhouse are presented in figure 6.

FIG. 6. Plants of (A) Cocozelle summer squash and (B) California Wonder pepper, disease-free and infected with the celery virus. These were inoculated by the use of viruliferous *Aphis gossypii*. In both cases the diseased plant is the one that appeared characteristically stunted and did not produce usable fruits of any sort. The healthy plants continued to grow vigorously and good fruits were harvested from them.

The proximity of occurrence and presence of the first diseased plants in fields in relation to original source of infection, were found to be almost perfectly correlated with distance and direction of travel by aphids walking over the ground away from infected border plants (Figs. 1, 2, 3, and 4). Wingless aphids have been observed many times, both in the field and in the greenhouse under cheesecloth cages, walking away from diseased plants for considerable distances and ascending healthy individuals upon which colonies became established and virus infection appeared. Winged forms of *Aphis gossypii* occur commonly in Florida but have never been found by the writer in great abundance around Sanford. The few times that flight has been studied were early in the morning, and the aphids in general flew towards the sun, though they were easily picked up by the easterly breezes and carried westward 25 to 150 feet from the plant from which they originally took flight. Moreover, the occurrence of virosis in widely scattered areas (Fig. 2, C and D), from which the trouble subsequently spread locally, has been found to occur at distances and in directions from original sources of infection that correlate well with the observed flights of winged aphids.

In the fields of plants studied, the celery virus spread most rapidly and extensively in Cocozelle and Golden Summer Crookneck squashes. In these

the plants averaged a little more than 9,500 to the acre. The rows were 36 inches apart with plants at about 18-inch intervals in the rows. Aphids multiplied very rapidly on these plants, soon became crowded, and travelled with readiness, carrying the virus across the field. The virosis also spread readily over pepper fields. These fields contained rows 30 inches apart with plants about 12 inches apart in the row, and approximately 17,000 plants per acre. Although this plant apparently is not so favorable a host for *A. gossypii* as is squash or celery, the aphids multiplied with ease and carried the celery virus from one part of the field to another. In celery there were around 60,000 plants per acre. The rows were 30 inches apart and the plants so close together (about 3 inches in many cases) that they soon made a solid and compact hedge of tender foliage in the row. Compactness of growth in celery was, perhaps, somewhat of a deterrent to spread of the celery virus, since aphids need go only a short distance to find an ample supply of leafy, vigorous, and succulent growth upon which to feed and multiply. In the cases of plants that have more space between them in the field, travelling aphids have to go farther, and have greater chance of passing between the plants as they go from one portion of the field to another. The spread of celery virus in maize (sweet corn) seemed to stop after the plants were about a foot high, when the aphids appeared to discontinue their feeding and went to other crops and bordering weeds.

THE NATURAL HISTORY OF *Celery Virus 1*

The celery virus is apparently endemic to the Florida peninsula. The writer found, with significant consistency, plants of *Commelina nudiflora* and *C. communis* L., infected with celery virus, growing wild, in many locations along river edges, in regions of the Everglades, and on the outskirts of hammocks miles away from habitations where it is impossible to conceive that, in every case, the plants were escapes from cultivation. In a recent trip to Cuba celery, squash, and pepper plants that appeared to be affected with typical virosis of celery, were found closely associated with virus-infected Commelina. Cook (2), in Puerto Rico, described and illustrated *C. longicaulis* Jacq. showing symptoms of what appeared to be typical celery virus. Kunkel (9) also has reported that, in Hawaii, *C. nudiflora* showed symptoms of virus infection.

Celery virus 1 occurs on several weeds in Florida, though in that region it has as its most important weed host *Commelina nudiflora* (*cf.* Doolittle (3) and Doolittle and Wellman (5)), as was proved by the writer through eradication of this weed alone (11), by which method it was possible to cut down spread of this virus to celery fields to a very marked extent. The virus exists in warm countries from season to season, over trying periods of extreme drought or rain, in the stems and buds of *C. nudiflora*. This

seed plant has no distinct season of quiescence in the form of seed or overwintering root stalk, but vegetates continuously and thus keeps the virus alive, and, in Florida, is not readily killed in nature by either excessively dry or wet weather.

Aphids act as vectors of the virus, carrying it from infected perennial plants of little or no apparent economic importance to those of annual type, where it multiplies and spreads vigorously. When the annual plants die or are chopped down, aphids leave and carry the virus back to nearby perennials, reestablishing themselves on old plants, or colonizing and infecting the young perennials, which act as reservoirs of the virus over periods unsuited to growth of the more tender plant species. It is upon the advent of new growth of many of the annuals that dissemination of the disease becomes most notable. The virus-carrying aphids migrate back from the less succulent and palatable reservoir hosts to annual crops and weeds, and the virus then becomes economically significant.

SUMMARY

The southern celery-mosaic virus (*Celery virus 1*) causes diseases that were found to spread in a generally characteristic manner in fields of various varieties of squash, pepper, and celery, and in the weeds *Commelina nudiflora* and *Ambrosia elatior*. It differs somewhat in its spread in maize.

The generally characteristic pattern of spread was studied in detail on celery. Initial appearance usually occurs on the edge of the field where there may be an infection source. This may be diseased weeds or other crop plants. The disease spreads inward through the successive development of small scattered areas that coalesce and, in turn, result in further infection and widely dispersed centers of disease. This may continue until the whole field is involved. In sweet corn the majority of infections occur in young seedlings toward the edges of the field next to infection sources. The disease spreads only while the corn is in the seedling stage.

There is some spread of this virus through mechanical means, but large increases in numbers of diseased plants in celery were observed to be dependent upon the occurrence of large numbers of the aphid vector, *Aphis gossypii*. Such a large proportion of plants becomes diseased that the virus, unless checked in its attacks, might well become a limiting factor in the production of certain cultivated host plants.

Observations showed that field dissemination depends largely on the movement of viruliferous aphids either in walking from plant to plant or in flying from one part of the field to another. The most rapid and extensive virus-disease spread occurred in squash fields and to a less extent in peppers. These plants are planted some distance apart in the field. In

fields of celery, which contain a great many more plants per acre, the disease was somewhat deterred in its spread by the excesssive amount of foliage encountered by the aphids.

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BUREAU OF PLANT INDUSTRY
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RELATION OF FUSARIUM AND HELMINTHOSPORIUM IN BARLEY SEED TO SEEDLING BLIGHT AND YIELD[1]

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INTRODUCTION

Barley seed blight is a disease complex that has received relatively little attention except when barley goes into market channels for malting or feeding purposes. As much of the barley produced in the Northwest is fed on the farm, seed blight often is overlooked. Recently, however, because of several outbreaks of scab, caused by *Gibberella saubinetii* (Mont.) Sacc., greater attention has been paid to the so-called blight of barley seed. Furthermore, the revived demand for good malting barley also has stimulated interest in the problem. The feeding value of barley would of course be considerably reduced if a large percentage of the blighted kernels were affected with *G. saubinetii*, which is likely to be toxic to pigs. The general impression that blighted barley is not likely to be very desirable for malting purposes also has intensified the problem. Under the circumstances there naturally is discrimination against blighted barley offered on the market for feeding or for malting purposes. There is another very important aspect to the problem from the farmer's standpoint. What is the value of blighted barley for seed? And can it be improved by seed treatment?

In Europe, seed disinfection is practiced extensively for the control of root rot of cereals, but in the United States very little has been done along this line. There are, however, rather conflicting reports on the effect of various fungicides on seed germination, vigor of growth, and yield. Therefore, in 1932 a study was begun on the relation of the microflora of barley seeds to germination, stand, vigor of growth, seedling blight, and the value of seed treatment. In 1934, the writers (4) published a preliminary note on the subject.

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EXPERIMENTAL RESULTS

Microflora of Barley Seed. During 1932–1933 the prevalence of Fusarium and Helminthosporium blight was determined for more than 900 samples of barley obtained from many different localities in the Northwest. In most cases 100 or more seeds were selected at random from each sample, dipped in 70 per cent alcohol, soaked in corrosive sublimate solution for 2 or 3 minutes, and then washed in a solution of 25 to 50 per cent Labarraque sodium hypochloride. The seeds were then placed on acidified potato-dextrose agar in Petri dishes. Many other methods of disinfecting the seeds were tried, but the above method was most satisfactory. Cultures were incubated at room temperature until colonies of fungi developing from the seeds were about 2 cm. or more in diameter. Identifications were then made (Fig. 1).

Several species of bacteria and fungi belonging to more than 25 genera were isolated. Alternaria was the most common fungus, both in 1932–1933 and 1933–1934. Species of this genus, therefore, can be considered the commonest cause of seed blight. Since it was shown, however, by extensive inoculations that *Fusarium* spp. and *Helminthosporium* spp. are the most important of the organisms isolated in causing seedling blight, particular attention was paid to these 2 genera, and the results given in this paper deal mostly with them.

Many different types of Fusarium were isolated, but *Fusarium graminearum* Schwabe was the predominant species. However, in 1933, *Fusarium* spp. belonging to section *sporotrichiella* were rather common. *Helminthosporium sativum* P. K. and B. was by far the most common species of Helminthosporium, although many other species were frequently isolated.

Naturally, there were marked variations in the percentage of seed infected with Fusarium and Helminthosporium. In 1932 the percentage of seeds infected with Fusarium (random selection) varied from 0 to 47 per cent, and Helminthosporium from 0 to 91 per cent. In 1933 the ranges were from 0 to 15 per cent and 0 to 53 per cent, respectively. Seeds with no conspicuous blemish often were infected with these 2 organisms. The average percentage of seeds infected with Fusarium and Helminthosporium in 308 samples in 1932 was 4.1 per cent and 15.1 per cent, respectively (Table 1). In 1933 it was lower, being 3.2 per cent for Fusarium and 13.8 for Helminthosporium, based on 276 samples. The results, given in table 1, also indicate that in 1932 and 1933 *Helminthosporium* spp. were more prevalent than *Fusarium* spp. on blighted seed. In general, seeds infected with Fusarium were infected with Helminthosporium also, the correlation coefficient between relative percentage of Fusarium and Helminthosporium infection for 250 samples in 1932 being 0.989 ± 0.004.

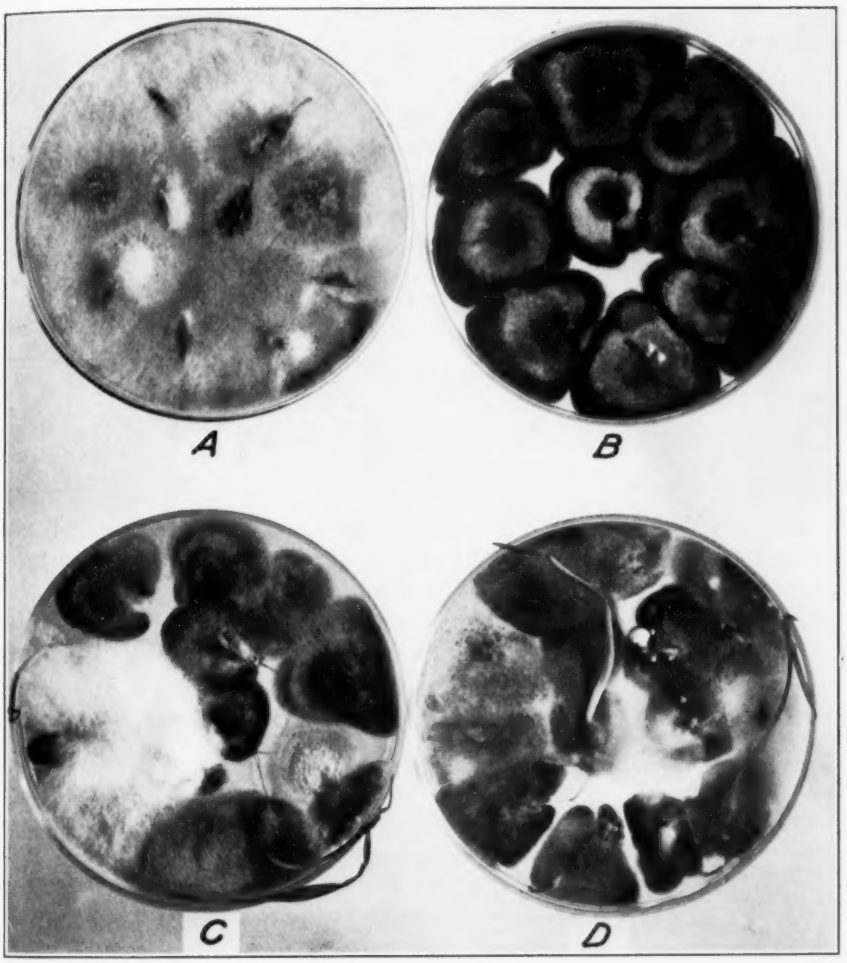

FIG. 1. Colonies of fungi developing from diseased barley seed. A and B. Artificially inoculated with *Fusarium* spp. and *Helminthosporium* spp., respectively. C and D. Naturally infected seed from the Northwest.

There were striking differences in prevalence of these 2 organisms in different regions in the Northwest. In 1932 isolations were made from several varieties, varying from 5 to 9, grown in 15 localities in 6 States. The relative prevalence of Fusarium and Helminthosporium for 10 localities for 3 representative varieties is given in table 2. (See also Tables 5 and 9). It is obvious from table 2 that, even within Minnesota, there were marked differences in percentages of seeds infected with Fusarium and Helminthosporium in different localities. Thus, at Waseca, Glabron had

TABLE 1.—*Prevalence of Fusarium spp. and Helminthosporium spp. on barley seed grown in different localities in the Northwest in 1932–1933*

Year and kind of seed	No. of samples	Percentage of infection			Ratio of Fusarium to Helminthosporium
		Fusarium	Helminthosporium	Total	
1932					
Random	308	4.1	15.1	19.2	1 : 3.7
Screenings	79	7.3	21.7	29.0	1 : 3.0
Blighted kernels	86	21.1	30.7	51.8	1 : 1.5
Blighted kernels	54	24.7	.....	.....	.....
1933					
Random	276	3.2	13.8	17.0	1 : 4.3
Blighted	141	7.4	22.3	29.7	1 : 3.0

30 per cent, Trebi 28 per cent, and Velvet 9 per cent infection; while at Morris, these same varieties had 5 per cent, 7 per cent, and 6 per cent infection, respectively. The cleanest barley tested in 1932 was obtained from western South Dakota and western North Dakota, while the most severely infected samples were obtained from Iowa and southern Minnesota. Neither Fusarium nor Helminthosporium was isolated from samples obtained from Dickinson, North Dakota, and Highmore, South Dakota, while

TABLE 2.—*The percentage of seeds infected with Fusarium and Helminthosporium in seed lots of 3 varieties of barley grown in 10 localities in 1932*

Place grown	Variety and percentage of infection[a]								
	Glabron			Trebi			Velvet		
	Fus.[b]	Hel.	Total	Fus.	Hel.	Total	Fus.	Hel.	Total
Crookston, Minnesota	3	5	8	1	8	9	0	1	1
Grand Rapids, Minnesota	2	7	9	6	27	33	2	13	15
Morris, Minnesota	2	3	5	2	5	7	0	6	6
Waseca, Minnesota	8	22	30	3	25	28	3	6	9
Fargo, North Dakota	1	2	3	0	4	4	0	5	5
Dickinson, North Dakota	0	0	0	0	0	0	0	0	0
Brookings, South Dakota	4	1	5	0	1	1	0	2	2
Highmore, South Dakota	0	0	0	0	0	0	0	0	0
Kanawha, Iowa	33	36	69	14	54	68	30	28	58
Ames, Iowa	33	25	58	6	91	97	23	25	58
Average	9	10	19	3	22	25	6	9	14

[a] Based on plating out seeds on nutrient agar.

[b] Fus. = *Fusarium* spp.; Hel. = *Helminthosporium* spp.

there was more than 50 per cent infection in those from Iowa (Table 2). Similar results were obtained also from a number of samples from southern Minnesota.

In 1933 the average percentage of barley kernels infected with Fusarium and Helminthosporium was lower than in 1932 (Table 1), although the infection extended over a wider area, and the total amount of seed blight was greater because of the very extensive infection with Alternaria, certain other fungi, and bacteria. Nevertheless, Fusarium and Helminthosporium were not uncommon, the latter being the more prevalent, as was true also in 1932.

Germination and Seedling Injury. In 1932 a study was made to determine the relationship between the percentage of seed infected with Helminthosporium and Fusarium and the following: germination, stand, root rot, seedling blight, stunting, and discoloration of the coleoptile (Table 3).

Germination and root-rot tests were made on moist blotters kept at 25° C. At the end of 7 days the degree of root-rot infection was observed. Seedling injury was studied in the greenhouse. The seed was sown in autoclaved sand in flats, 6 rows of 100 seeds each being sown in each flat. The temperature ranged from 20° C. to 30° C., which is favorable for infection with Fusarium and Helminthosporium. Notes were taken approximately 3 weeks after planting. The results are summarized in tables 3 and 4.

The negative correlation coefficient between percentage of seeds infected and percentage of germination, namely -0.331 ± 0.056, indicates that the higher the percentage of Fusarium and Helminthosporium infection, the poorer the germination. Although there was a significant negative correlation between the degree of infection and the percentage of germination, the difference in germination between clean seed and infected seed often is so slight that this criterion alone is not always a reliable index of the value of the sample for seed. The development of the seedlings also must be taken into consideration, except, of course, in the case of very badly blighted samples.

Special germination tests were made of 118 samples of distinctly blighted seed at 16° C., the temperature of the malting floor. Approximately 69 per cent of the diseased seed germinated, 50 per cent normally, the other 19 per cent weakly or abnormally. The primary rootlet of such seedlings often was stunted, rotted, or aborted; the plumule of some seedlings was contorted, short or even absent, the roots and plumule of others were both injured (Figs. 2 and 3). Ninety-three per cent of the sound seeds selected from these seed lots germinated and nearly all developed strong shoots.

Many of the retarded or weak seedlings that developed on blotters would not have emerged from the soil, while others probably would have been killed a few days after they emerged. This is indicated by the fact that the

TABLE 3.—*Effect of sowing barley seed with different percentages of Helminthosporium and Fusarium infection on germination and seedling injury*[a]

Percentage of infection			Percentage		Number of plants			Degree of root rot
Fusarium	Helmintho-sporium	Total	Germination on blotters	Emergence from sand	With discolored coleoptile	Stunted	Killed	
0	0	0	99	98	11	6	1	10[b]
0	1	1	90	88	3	18	0	30
1	4	6	94	95	14	11	0	10
2	3	4	96	93	3	9	0	10
2	5	7	97	95	24	9	0	10
9	2	11	94	88	16	14	4	30
4	20	24	96	82	37	14	7	40
14	22	36	97	90	24	46	15	50
7	31	38	93	84	38	40	10	40
23	25	48	92	84	28	38	8	50
12	36	48	91	85	18	72	7	80
30	28	58	97	84	50	41	20	30
4	63	67	95	87	11	80	7	60
25	45	70	81	70	59	17	8	30
18	64	82	90	80	37	79	8	90
15	68	83	91	67	29	27	5	70

[a] These are representative samples of approximately 300 barley lots.

[b] Numerals 0–100, in ascending order, indicate relative degree of root-rot infection.

FIG. 2. Germination of blighted seed. Note discoloration of roots and coleoptiles.

average percentage of germination on blotters[3] in 213 samples was 94.5 per cent, but the stand for the same samples sown in sand was only 88 per cent. A correlation coefficient of 0.332 ± 0.068 was obtained between degree of root rot and number of plants stunted. Root infection also is correlated with seed infection ($r = 0.394 \pm 0.065$). This tends to indicate that severity of root infection on blotters is a fairly good index of the development of root rot in soil. Such tests, however, to be reliable, must be made at a relatively high temperature, preferably above 20° C. Many tests were made on blotters at 16°, 20°, and 25° C. Although the seeds were heavily infected with Fusarium and Helminthosporium, practically no root rot developed at 16° C., while at 25° C. the same samples developed severe root rot.

The negative correlation coefficient (-0.507 ± 0.046) between percentage of seeds infected and percentage of plants that emerged proves that Fusarium and Helminthosporium are important in lowering the vitality of barley seed. As one might expect, a high correlation, 0.490 ± 0.048, was obtained between the percentage of germination and percentage of plants that emerged.

[3] The writers are indebted to Mrs. Ruby U. Crouley, Seed Analyst, State Seed Testing Laboratory, University Farm, St. Paul, Minnesota, for making the germination tests reported on blotters, and to Mr. John L. Larson, Assistant Seed Analyst, for his efficient help in the special germination studies.

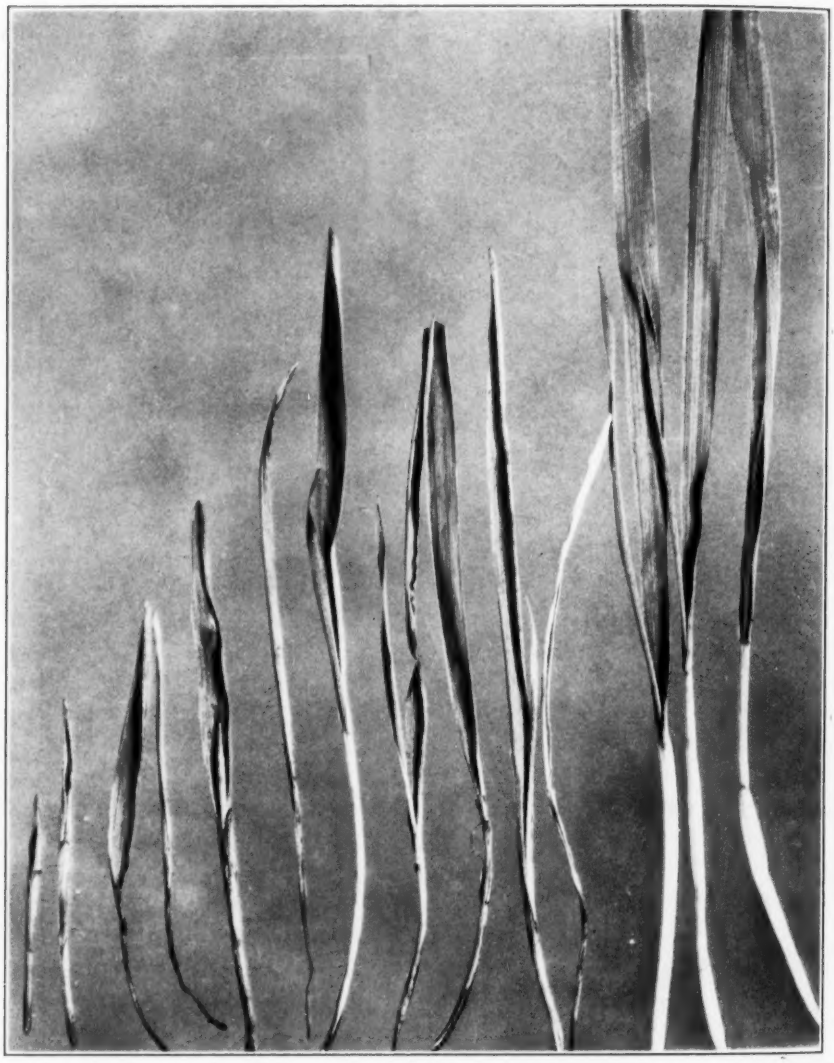

FIG. 3. Diseased seedlings from seed infected with Fusarium and Helminthosporium; three healthy ones on right.

The data given in tables 3 and 4 also indicate that the percentage of seeds infected with Fusarium and Helminthosporium is closely correlated with the following: severity of root-rot infection, (0.394 ± 0.065), discoloration of coleoptile, (0.376 ± 0.057), and number of plants killed, (0.313 ± 0.039). The results indicate that the amount of seedling injury is proportional to the percentage of seeds infected by the 2 pathogens.

When the percentage of seeds infected with Fusarium or with Hel-

TABLE 4.—*Correlation between quality of seed and germination and subsequent development of seedlings*

Number samples compared	Data correlated		Correlation coefficient[a]
250	Percentage seed infected with Fusarium	Percentage infected with Helminthosporium	0.989 ± 0.004
213	Percentage seed infected with Fusarium and Helminthosporium	Percentage seed that germinated	−0.331 ± 0.056
213	do	Percentage plants that emerged	−0.507 ± 0.046
213	Percentage seed that germinated	do	0.490 ± 0.048
116	Percentage seeds infected with Fusarium and Helminthosporium	Degree of root-rot infection (on blotters)	0.394 ± 0.065
234	do	Number of plants with discolored coleoptile	0.376 ± 0.057
234	do	Number of plants stunted or contorted	0.402 ± 0.055
234	do	Number of plants killed	0.313 ± 0.039
234	Percentage seeds infected with Fusarium	Number of plants stunted or contorted	0.288 ± 0.060
234	Percentage seeds infected with Helminthosporium	do	0.382 ± 0.051
168	Degree root-rot infection (on blotters)	do	0.332 ± 0.068

[a] According to Fisher's values for levels of significance of correlation coefficients, all the correlation coefficients surpass the 1 per cent point; therefore, they are highly significant.

minthosporium alone was compared with the number of plants stunted or killed, correlation coefficients of 0.288 ± 0.060 and 0.382 ± 0.051, respectively, were obtained. These figures are lower than that obtained for their combined effect (0.402 ± 0.055). The results indicate that Helminthosporium is more destructive to seedlings than Fusarium, although the correlation coefficient for the latter may have been higher than that obtained had as many seeds been infected with Fusarium as with Helminthosporium. The ratio of the former to the latter in random samples was about 1:4 (Table 1).

Seed treatment. As much of the barley seed produced in Minnesota in many years is likely to be infected with Helminthosporium, Fusarium, and other fungi that cause seedling injury, the effect of seed treatment on the development of seedling blight was tried. Ceresan was selected as the disinfectant because it eliminates barley stripe and might, therefore, be expected to kill other fungi in the glumes and seed coats. The tests were made on 3 varieties of barley from 7 localities. The treated seed was sown

TABLE 5.—*The results of Ceresan treatment of barley seed obtained from seven localities and infected with different amounts of Fusarium and Helminthosporium, when sown in sand in the greenhouse, 1932*

Variety and source of seed	Percentage of seed infected		Percentage emergence		Number of plants							
					Killed		Stunted		Discolored coleoptile		Primary leaf spots	
	Fus.[a]	Hel.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.
Glabron, Minn. No. 445												
Dickinson, North Dakota	0	3	95	94	1	0	5	2	14	0	2	0
Waseca, Minnesota	8	22	82	83	3	0	18	4	45	7	14	0
Winnebago, Minnesota	47	4	77	88	18	3	41	9	82	29	8	1
Ames, Iowa	23	61	74	82	9	2	30	13	77	24	20	7
Minsturdi, Minn. No. 439												
St. Paul, Minnesota	1	3	95	97	1	0	6	3	17	2	2	0
Ames, Iowa	18	64	80	86	13	1	37	6	80	20	21	4
Velvet, Minn. No. 447												
Morris, Minnesota	0	6	88	91	2	1	9	5	15	4	3	0
Waseca, Minnesota	3	6	80	83	13	1	35	8	80	5	14	1
Marshall, Minnesota	32	22	69	84	25	1	55	9	86	21	25	2
Ames, Iowa	23	25	78	81	13	2	41	17	80	4	19	2
Average	16	22	82	87	10	1	28	8	58	12	13	2

[a] Fus. = Fusarium; Hel. = Helminthosporium; Ck. = Check; Tr. = Treated.

in triplicate rows in sterilized sand in flats in the greenhouse. Results are given in table 5.

Ceresan treatment materially increased the stand and decreased number of plants killed, stunted, with discolored coleoptile, and the number with primary lesions. The value of this treatment was associated closely with the degree of seed infection. The higher the percentage of seed infected, the more beneficial the treatment. There was little or no value in treating practically clean seed, *i.e.*, Glabron from Dickinson, North Dakota, or Minsturdi from University Farm, St. Paul. One hundred and eight samples selected at random from the 1933 crop were tested in similar manner except that only a single test was made of each lot. Forty-seven per cent responded to Ceresan treatment and there was no appreciable effect on the remainder.

Most of the seed lots that were not improved by treatment were virtually free from Fusarium and Helminthosporium, although many were heavily infected with other fungi, especially Alternaria. An additional 15 samples of barley free from Fusarium and Helminthosporium and obtained from Washington and Montana also were treated with Ceresan. Not a single sample responded to the treatment. There seems to be little value in treating clean seed if sown in clean soil.

The beneficial effect of treating infected barley seed becomes even more evident when the green weight of seedlings obtained from treated and nontreated seed are compared (Table 6). In this test the barley plants were

TABLE 6.—*Green weight of seedlings grown from treated and nontreated barley seed with different percentages of Fusarium and Helminthosporium infection*

Variety	Per cent seed infected	Mean weight in grams		Per cent increase over check	Differences between means in grams	Differences between means divided by probable error of difference
		Check	Treated[a]			
Glabron	3	11.17 ± 0.32	12.03 ± 0.11	8	0.85 ± 0.34	2.5
do	30	8.60 ± 0.23	12.10 ± 0.48	41	3.50 ± 0.83	4.2
do	50	7.13 ± 0.19	11.97 ± 0.10	68	4.84 ± 0.54	8.9
do	64	6.90 ± 0.03	10.33 ± 0.19	50	3.43 ± 0.19	17.9
Minsturdi	4	15.0 ± 0.52	17.57 ± 0.37	17	2.57 ± 0.64	4.0
do	82	6.17 ± 0.28	13.20 ± 0.56	114	7.03 ± 0.63	11.2
Velvet	6	10.40 ± 0.23	10.87 ± 0.37	5	0.47 ± 0.43	1.1
do	9	6.63 ± 0.25	11.00 ± 0.22	66	4.37 ± 0.32	13.8
do	52	5.63 ± 0.14	10.07 ± 0.20	79	4.44 ± 0.24	18.8
do	48	6.87 ± 0.13	10.63 ± 0.12	55	3.76 ± 0.17	21.7

[a] Treated with Ceresan 3 oz. per bu.

cut off at the ground line when about a month old and weighed immediately. The samples were the same as those listed in table 5. The greatest increases in weight over the checks were obtained from plots sown with the heavily infected seed. For instance, Minsturdi, with 82 per cent of infected seeds, produced 114 per cent more green weight than the control; with 4 per cent infection, the increase was 17 per cent. In both cases the increase was statistically significant. Treating Glabron with 3 per cent and Velvet with 6 per cent infection caused no significant increase in the green weight, but when the percentage of infection was higher there was significant increase.

After obtaining such striking results in the greenhouse, similar tests were made in the field. One hundred and sixteen samples of barley, representing a dozen or more varieties, were selected because of wide variation in degree of infection with Fusarium and Helminthosporium. One portion of the seed was treated with Ceresan, the other was used as control. The treated and nontreated seed was sown in adjoining rows in duplicate series. The test was made on sandy land, previously cropped to rye, near Anoka, Minnesota. Comparative notes on the stand and vigor of growth were taken when the seedlings had developed 2 or 3 leaves and again 3 weeks later. The final percentage figures were based on the average estimates of 3 observers. As each sample had been assigned a code number, all readings were made without knowledge of the kind of seed used. The data thus obtained were correlated with the following: (1) percentage of seeds infected with Fusarium; (2) percentage of seeds infected with Helminthosporium; (3) percentage of seeds infected with both; (4) percentage of plants that emerged in greenhouse; (5) degree of root rotting as determined on moist blotters; (6) number of plants stunted or deformed; and (7) bushel weight of sown grain. The results are summarized in table 7.

All the correlation coefficients listed in table 6 are highly significant except that for bushel weight (0.016 ± 0.093). The results agree in general with those obtained in the greenhouse. They show clearly that poor stand and lack of vigor of seedlings are distinctly associated with increase in percentage of infection in the seed sown. This is particularly noteworthy because the seed was sown in soil cropped the previous year to rye; hence "soil infection" would be expected. But in this experiment seed infection was far more injurious than soil infection. This may be explained, at least partially, on the basis of recent investigations by Henry (5), Sanford and Broadfoot (7), and Bisby, James, and Timonin (1), who have shown that antibiosis of microorganisms of the soil plays a very important rôle in preventing root-rot infection. Preliminary tests at Minnesota indicate that the soil flora has very little effect on preventing root rot and seedling blight resulting from naturally infected seed. It is significant that lightweight, clean seed is better than heavy, infected seed (Tables 6 and 7).

TABLE 7.—*The effect of treating different seed lots of barley with Ceresan, as shown by correlation between the degree of infection in the treated seed and its performance when sown in the greenhouse and field*

Stand and vigor of growth resulting from Ceresan treatment compared with—	Correlation coefficient
Bushel weight	0.016 ± 0.093[a]
Percentage seeds infected with Helminthosporium	0.834 ± 0.028
Percentage seeds infected with Fusarium	0.580 ± 0.061
Percentage seeds infected with Fusarium and Helminthosporium	0.826 ± 0.029
Percentage of plants that emerged	−0.456 ± 0.073
Degree of root infection (on blotters)	0.280 ± 0.084
Number of plants stunted	0.348 ± 0.081

[a] Not significant according to Fisher's values for levels of significance of correlation coefficients, but all the others (correlation coefficients) surpass the 1 per cent point; therefore, they are highly significant.

It seems significant that the correlation between percentage of seeds infected with Helminthosporium alone and the effect of seed treatment was as high (0.834 ± 0.028) as that for combined percentages of Fusarium and Helminthosporium infection and the effects of treatment (0.826 ± 0.029). The correlation between Fusarium infection and the effect of treatment with Ceresan was 0.580 ± 0.061. These high correlations prove that both Fusarium and Helminthosporium are very important in reducing stand and vigor of growth of barley.

Obviously, the kind of seed is important, but only with respect to the degree of infection with fungi (Fig. 4). Table 8 gives the increase in stand and vigor of growth resulting from seed treatment for 7 varieties of barley obtained from 6 localities in Minnesota, 2 in North Dakota, and 2 in Iowa. In Minnesota, the seed lots that responded least to the treatment were from Morris (2.1 per cent infection), while greatest stimulation was in seed from Waseca and Grand Rapids (21 per cent and 19 per cent infection, respectively). The greatest effect of all the treatments was for barley from Ames, Iowa. The 5 varieties of barley from this locality had an average infection of 53 per cent, and the average stand and vigor of growth was increased 54 per cent by the treatment.

The favorable effect of Ceresan on seedlings of barley led to yield trials. Ten different lots of barley with marked variation in percentages of infection with Fusarium and Helminthosporium were treated with Ceresan. The tests were made in 5 sets of duplicate rows, 18 feet long, every other

FIG. 4. The relative vigor of seedlings of barley grown from diseased and relatively clean seed. Left, seedlings from seed with 21 per cent infection; right, from seed with 4 per cent infection (Fusarium and Helminthosporium).

TABLE 8.—*The effect of Ceresan on barley seed with different percentages of Fusarium and Helminthosporium infection when sown in the field in 1933*

Variety	Source percentages of infection, vigor, and stand over checks																			
	Waseca, Minn.		Gr. Rapids, Minn.		St. Paul, Minn.		Duluth, Minn.		Crookston, Minn.		Morris, Minn.		Fargo, N. D.		Dickinson, N. D.		Ames, Iowa		Kanawaka, Iowa	
	Inf.[a]	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.
Glabron	30	0	0	0	7	0	8	0	8	0	0	0	10	0	0	15	84	60	69	40
Manchuria	26	15	18	25	10	55	5	40	4	0	0	0	4	0	0	0	76	95	65	75
Peatland	22	30	10	20	33	0	8	15	5	0	9	0	.....	.....	.....	.....	.....	.....	.....	.....
Svansota	51	35	28	45	7	0	8	0	9	50	4	0	8	0	0	0	.....	.....	.....	.....
Trebi	28	50	33	0	4	10	16	15	9	10	7	0	4	10	0	10	97	105	80	95
Velvet	9	0	15	0	20	20	4	0	1	10	6	15	5	0	0	0	48	50	30	50
Wis. No. 38	26	20	18	15	9	0	11	25	5	10	6	0	6	0	0	0	67[b]	65	20[b]	45
Average	27.4	21.4	18.7	15.0	12.9	12.1	8.6	13.6	5.9	11.4	4.6	2.1	5.3	1.4	0.0	3.6	53.1	53.6	37.7	43.6

[a] Inf. = Percentage of seeds infected with Fusarium and Helminthosporium; Inc. = percentage increase, based on relative stand and vigor of growth in comparison with nontreated seed.

[b] Wis. No. 37 instead of Wis. No. 38.

row being a border. At harvest time 1 foot from each end of the row was discarded. The weight was determined to the nearest gram. The data were analyzed statistically by Student's method. The results are given in table 8. Yields were increased materially by Ceresan treatment, whenever seed lots were heavily infected, except for the variety Glabron. The yield of Velvet from seed with 54 per cent infection was increased 23 per cent, while that of Minn. No. 457 with 21 per cent infection was increased 20 per cent. In general, the percentage of increase resulting from treatment was proportional to the severity of infection. Therefore, the increase probably was due to the effect of Ceresan on the seed-borne pathogens, which were

TABLE 9.—*Yields produced by different seed lots of barley treated with Ceresan and sown in quintuplicate 18 foot rows in the field at University Farm, St. Paul, Minnesota, in 1933*

Kind and source of seed lot	Percentage seeds infected			Increase or decrease in yield per acre[a]		Z[b]	Odds
	Fus.	Hel.	Total	Bu.	Pct.		
Glabron, Minn. No. 445							
St. Paul, Minnesota	2	5	7	+ 0.8	+ 4.5	.58	5
Waseca, Minnesota	8	22	30	+ 0.1	+ 0.7	.09	<1
Winnebago, Minnesota	47	4	50[c]	+ 0.3	+ 1.1	.62	6
Manchuria, Minn. No. 184							
St. Paul, Minnesota	2	8	10	− 0.3	− 2.9	.63	6
Waseca, Minnesota	3	24	26[c]	+ 1.6	+15.1	2.96	500
Minn. No. 457							
St. Paul, Minnesota	1	3	4	+ 1.3	+ 4.9	1.9	103
Waseca, Minnesota	9	12	21	+ 5.5	+20.2	12.4	>10,000
Minsturdi, Minn., No. 439							
St. Paul, Minnesota	1	3	4	+ 0.1	+ 0.4	.09	<1
Velvet, Minn. No. 447							
St. Paul, Minnesota	4	16	20	+ 3.3	+18.2	2.5	275
Waseca, Minnesota	3	6	9	+ 3.6	+19.5	6.9	>10,000
Marshall, Minnesota	32	22	52[c]	+ 4.6	+23.4	9.8	>10,000

[a] As compared with nontreated checks of same seed lot.

[b] Analysis made according to Student's method.

[c] Fusarium and Helminthosporium colonies grew from same seed.

destroyed or at least checked effectively by the fungicide. O'Brien and Dennis (6) obtained similar results with oats infected with *Helminthosporium avenae* in Scotland.

One formaldehyde dust also was tried, but either no increase or an actual decrease in yield resulted. In 1923–1924, a series of tests were made at University Farm on the merit of seed treatment with several fungicides (Ceresan not included) on 4 varieties of barley (3). None increased or decreased the yield. In the light of the results of the present investigations, the negative results may be attributed to the use of comparatively clean seed or ineffective fungicides. It will be noted from table 8 that the yields are rather low. In 1933, it was unusually dry and the apparent differences early in the season between treated and nontreated seed, especially in 2 lots of Glabron, were not substantiated by significant differences in yield. Differences in yield between treated and nontreated seed might be greater in a season of normal rainfall.

The behavior of Glabron toward seed treatment is puzzling. Tisdale, Taylor, and Griffiths (8) concluded that barley varieties are affected differently, with respect to yield, by treating the seed with fungicides. Burnett (2) found that treating seed of Velvet and Glabron with Ceresan did not increase yields, while it increased that of certain other varieties considerably. Under our conditions, however, Velvet responded to seed treatment but Glabron did not.

DISCUSSION

Barley grown in the Northwest is commonly attacked and frequently discolored by a large group of fungi and bacteria. Seeds heavily infected usually are discolored, shrivelled, and of inferior quality. Seedling blight, root rot, basal stem rot, and decrease in yield frequently result when such seed is sown. *Fusarium* spp. and *Helminthosporium* spp. are the most virulent pathogens usually associated with the blighted kernels and with seedling blight.

The results of extensive plating tests show that fungi belonging to more than 25 genera, as well as several kinds of bacteria, cause seed blight in barley. In general, *Helminthosporium* spp. and Alternaria often are more responsible for blighting of the seed than Fusarium. In 1932, for example, Helminthosporium was between 3 and 4 times as prevalent as Fusarium in lots of barley obtained principally from Minnesota and the Dakotas. In 1932 and 1933 Alternaria and bacteria were far more prevalent than either Helminthosporium or Fusarium. It is evident, therefore, that the blighting of barley seed in the spring-wheat region is caused by a group of organisms and that field surveys and plating tests must be resorted to in order to ascertain the relative amount of scab and of blight caused by fungi other than that or those producing the dangerous scab.

Relatively little attention has been paid to the control of the disease complex known as "blight." In fact, relatively little has been known about the suitability of blighted barley for seed.

Laboratory, greenhouse, and field experiments show clearly that there is high correlation between the percentage of seeds infected with *Helminthosporium* spp. and *Fusarium* spp. and the percentage of germination, the stand, the amount of root rot, the number of stunted and deformed plants, and discoloration of the coleoptile. Blighted barley, therefore, is not good seed barley. In some years, however, such as 1933, it would be difficult to obtain good, clean seed barley; hence the question of the effect of seed treatment becomes important.

Treating blighted barley seed may be very beneficial. In the experiments reported in this paper the value of treatment depended on the degree of blighting in the seed, the variety in question, and the fungicide used. As might be expected, the value of treatment was directly proportional to the percentage of infected kernels in the seed lot. For some unknown reason there was no appreciable beneficial result from treating even badly infected seed of Glabron barley. There seem, therefore, to be decided differences in the effect of fungicides on different varieties. Of the fungicides used, Ceresan produced the best results. Formaldehyde dust, on the other hand, not only did not improve stands, but even depressed yield in some cases.

The fact that treating seed with Ceresan usually resulted in better stands and more vigorous plants and that the improvement was proportional to the degree of blighting of the seed indicates that this fungicide, as well as others of the same nature, probably brings about its "stimulatory" effect by killing or inhibiting the deleterious microflora of the seed. It should be emphasized, however, that seed treatment does not always result in increased yields. There are several variables to consider and there may be no beneficial effect from treatment. It is quite clear, however, that yields can often be increased considerably by treating blighted seed or, preferably, by sowing clean seed if it is available.

SUMMARY

During 1932–1934 a study was made of the relative prevalence of Fusarium and Helminthosporium in a large number of samples of Northwestern barley grown in 1932 and 1933 and of the effect on the succeeding crop.

In 1932 Helminthosporium was 3 to 4 times more prevalent than Fusarium. In 1933 the percentage of infected seed was less, but the ratio of Helminthosporium to Fusarium was slightly higher.

In some regions barley was relatively free from "seed blight" in 1932 but was severely blighted in 1933. But most of the discoloration was not primarily due to Helminthosporium and Fusarium but to bacteria and other fungi, especially Alternaria.

Fusarium and Helminthosporium were the common virulent root-rotting organisms associated with diseased barley seed.

There is high correlation between percentage of seed infected with Helminthosporium and Fusarium and the percentage germination of seed, stand, amount of root rot, seedling blight, the number of stunted or deformed plants, and discoloration of coleoptile.

Treating infected seed with Ceresan resulted in significantly better stands and more vigorous growth of seedlings, the improvement being approximately proportional to the degree of infection of the treated seed. Benefits derived from other treatments varied with the severity of seed infection and the fungicide used.

There was no significant correlation between bushel weight of treated seed and the effect of Ceresan treatment.

Seed treatment with Ceresan increased yields, except when the seed was practically clean or when Glabron was used.

There appear to be distinct varietal differences in behavior of barley toward seed treatment, at least as far as yield is concerned. Glabron, although sometimes severely infected, did not respond to seed treatment, while Velvet, Manchuria, and others did.

Infected barley seed treated with Ceresan is not so good as clean, healthy seed.

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A DESCRIPTIVE KEY FOR PLANT VIRUSES

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Research on plant viruses has reached a stage where it is both a challenge and a duty to science to organize such lines of endeavor as can never be strictly a matter of individual concern. The related problems of description, identification, nomenclature, and classification, though they may be particularly difficult subjects upon which to secure general agreement, are, partly for that very reason, in special need of correlated thought and effort. Despite the generally recognized need for an accepted system of description and nomenclature in particular, it appears to be the consensus of opinion that early agreement on such a system is not likely to be secured.

The difficulties in these related problems arise partly from the confusion already existing and partly from the apparently increasing complexity of the subject as further knowledge is gained. The number of distinct plant virus diseases recorded in the literature already exceeds one hundred, and these involve approximately seven hundred plant species. One hundred or more workers are now engaged in the field of plant-virus research in various parts of the world, and there is good reason to believe, therefore, that the number of viruses and hosts reported will continue to increase rapidly for some time. The danger of adding to the present confusion through delay in applying some acceptable system of description is consequently a matter that warrants serious consideration.

Judging from the experience of systematists in other biological fields, the best procedure for a time may be for individuals to contribute such specific suggestions and ideas as they may have on the subject, while at the same time aiming at some form of general agreement through national and international cooperation. It is with this thought and purpose in mind that a tentative scheme for a descriptive key for plant viruses has been drawn up, which is presented and discussed in the following pages. Its greatest value may eventually lie in illustrating the possibilities for the development of a uniform system of description, and in focusing further attention and effort on the problem.

HISTORICAL ASPECTS

The early descriptions of plant virus diseases were naturally chiefly concerned with characteristics that would serve to separate these peculiar maladies from diseases of plants due to other causes. The time had not yet come for attention to distinctions between individual virus diseases. Indeed, the idea that a single type of causal agent might be concerned with

all known forms of plant virus disease prevailed for a considerable time, and on this assumption such distinctions as were noted were accounted for on the basis of the host affected. Even after the description of several different forms of virus disease on a single host (8), true recognition of the existence of a number of specific viruses came only very slowly. This is not surprising in view of the absence of any morphological grounds on which to differentiate the causal agents, and the reliance that was placed on symptomatology for descriptive purposes. The difficulties of differentiation on this basis increased rapidly with the discovery of the influence of host variety and of environmental factors on symptom expression, which, together with complications in terminology and nomenclature, often made identification of a particular disease impossible or uncertain. With the more recent discovery that certain of the better known virus diseases are actually due to the combined action of two or more different viruses, little hope could be maintained for much progress in separating the increasing number of diseases recognized on the criterion of symptoms alone.

Consequently, although the differentiation of virus diseases such as those of the potato on the basis of symptom expression served its purpose very well in the earlier periods of study, it now appears that this method is of only limited application to plant virus diseases as a group. Aside from the specific criticisms of inadequacy and unreliability, which may be offered against this system, the principle itself is faulty from the point of view of modern pathology. It is to be regretted, therefore, that much of the more recent confusion in literature on virus diseases is attributable partly to their continued description on the basis of symptomatology where such appears to be no longer necessary nor desirable.

Description and identification of the virus itself, whenever possible, on the basis of its behavior and reactions (4, 5, 6, 9) offers far greater opportunities than is generally suspected, and is furthermore likely to remain the only satisfactory means of differentiation. This principle has now been given sufficient trial to warrant general assurance of its reliability, and to a considerable extent has been found adequate. Adoption of the principle of describing the virus rather than the disease does not eliminate the usefulness of symptom descriptions as a valuable adjunct, but merely reduces the emphasis on this feature in cases where it is neither reliable nor necessary. The plan presented in this paper for the description of plant viruses proposes, in fact, to use for differential purposes any or all characteristics of the virus or of the resultant disease according as need arises.

THE BASES FOR DESCRIPTION OF A PLANT VIRUS

From the point of view of identification, the simplest and most concise description that will suffice to distinguish one virus from any other will

prove the most satisfactory. Those who are acquainted, through repeated observation or research, with a particular virus disease may regard even a very elementary description of the virus itself as superfluous, since the disease symptoms may be regarded as entirely sufficient for recognition. This criticism, however, may have application to only relatively few viruses, and, even in such cases, a fundamental description may often serve to place specialists in a particular virus on guard for new forms. Certainly, a long acquaintance with ordinary tobacco mosaic would not qualify anyone to make a reliable determination by inspection only.

On the other hand, the introduction of minute details of behavior or properties of a virus, which are unnecessary or determinable only with difficulty, should not be included in a description merely because these points are known. Nevertheless, such features may eventually become significant for descriptive purposes. For the present, however, it has not been found necessary or advisable to utilize such characteristics as filterability, tolerance to dilution, resistance to chemicals, serological reactions, adsorption relations, influence of environment on symptoms, distribution of virus in host, or many other details of changes of a macroscopic or microscopic character.

In the light of present knowledge, the features of paramount value for the description and identification of plant viruses are considered to be: (1) Modes of transmission, including the identity of any known insect vector; (2) natural and differential hosts; (3) longevity *in vitro*; (4) thermal death point; (5) certain distinctive or specific symptoms.

The various modes of transmission of a plant virus, in addition to forming distinctions of major importance, may also serve for more specific purposes. This is particularly true of transmission by sucking insects, for not only does the type of insect, *i.e.*, aphid, leaf hopper, thrips, white fly, form an important distinction, but the specific vector concerned is in many instances the main criterion for identification. Transmission by plant extract, tissue insertion, or grafting, or through vegetative parts, seed, or soil complete the possible means by which a virus may be spread. The use of natural or differential hosts as a means of determination constitutes a useful but often less reliable criterion than the modes of transmission, unless considered in conjunction with insect relations, virus properties, or specific symptoms.

DEVELOPMENT OF THE KEY

When the various characters discussed above are established, within the limits of possibility, for individual viruses, it becomes possible by different methods to separate the viruses into several fairly well-defined groups. As these are studied in more detail, it becomes apparent that some degree of

natural relationship exists within certain groups. Hence, in the further development of such groupings into a descriptive key for plant viruses, the question of possible natural relationships must influence the choice of the system to be followed. Furthermore, in the creation of divisions and subdivisions of the different virus groups in a dichotomous system to form such a key, great importance must be attached to the criteria or bases on which the first distinctions are made. The relationship of sucking insects to plant viruses constitutes perhaps the most natural and fundamental line of separation (1, 6, 9). Thus the viruses may be separated first into those that are, and those that are not, transmissible by some type of sucking insect. The latter group, depending as it does on negative results of experimental tests, may, to be sure, eventually prove to contain many viruses that must be transferred to the former group, and it may finally develop, as is now suspected by some, that all viruses are transmissible by some sucking insect. In such event, this first criterion for separation will naturally be eliminated and the subsequent criteria, especially the identity of the vector involved, will increase in relative significance.

Everything considered, it has seemed most satisfactory to base the second criterion of distinction on mechanical transmissibility of the virus by plant extract. It might be maintained that second rank should be allotted to the type of insect vector concerned. Nevertheless, it is considered preferable to sacrifice this choice for the desired sequence in subsequent subdivisions, intended to be based largely upon certain properties of the viruses *in vitro*. Accordingly, the identity of the insect vector has been used as a subsequent criterion of differentiation, and it attains special significance in those subgroups where mechanical transmission by plant extract fails. For the convenience of the reader, the chief subdivisions used in the key are shown in schematic form in table 1.

In mechanical transmission by plant extract, two criteria are involved, namely, that plant extract must yield infection and that infection must be accomplished by simple or ordinary methods of inoculation, such as needle punctures, rubbing, or injection. Mechanical insertion of pieces of tissue, with or without union of cells, and infection from plant juice through the medium of insects have been excluded from this category. For the present, also, it has seemed advisable to exclude all rare, doubtful, or very difficult cases of transmission by means of plant extract. Separate groups for viruses transmitted by more unusual methods, such as the insertion of pieces of tissue (as distinguished from true grafting), have not been included, because these modes of transmission are not regarded as fundamental in importance, nor are they always easily accomplished with certainty.

The striking range in longevity *in vitro* and in thermal death point of viruses that are transmissible mechanically by plant extract has suggested

TABLE 1.—*The chief groups of plant viruses*

The Plant Viruses	Transmissible by sucking insects (A_1)	Not transmissible mechanically by plant extract (B_1)	Not transmissible by aphids (C_1)	Transmissible by leaf hoppers (D_1)
				Not transmissible by leaf hoppers (D_2)
			Transmissible by aphids (C_2)	Rosaceae susceptible (D_1)
				Rosaceae not susceptible (D_2)
		Transmissible mechanically by plant extract (B_2)	Longevity *in vitro* less than 7 days at 22° C. (C_1)	Thermal death point below 60° C., 10 min. (D_1)
				Thermal death point 60° C., or above, 10 min. (D_2)
			Longevity *in vitro* 7 days or more at 22° C. (C_2)	Thermal death point below 80° C., 10 min. (D_1)
				Thermal death point 80° C., or above, 10 min. (D_2)
	Not transmissible by sucking insects (A_2)	Transmissible mechanically by plant extract (B_1)	Longevity *in vitro* 7 days or more at 22° C. (C_1)	Thermal death point 60° C., or above, 10 min. (D_1)
				Thermal death point less than 60° C., 10 min. (D_2)
			Longevity *in vitro* less than 7 days at 22° C. (C_2)	Thermal death point 60° C., or above, 10 min. (D_1)
				Thermal death point less than 60° C., 10 min. (D_2)
		Not transmissible mechanically by plant extract (B_2)	Transmissible by grafting (C_1)	Potato susceptible (D_1)
				Potato not susceptible (D_2)
			Not transmissible by grafting so far as known (C_2)	Transmissible by other vegetative means (D_1)
				Not transmissible by other vegetative means (D_2)

the employment of these features in the differentiation of certain groups. Some degree of natural relationship appears to be reflected in the behavior of certain viruses toward these tests; though the points of separation selected in the present scheme are, of course, purely arbitrary.

Separation on the basis of longevity of extracts *in vitro* offers many possibilities. For the sake of simplicity and convenience, however, only two subdivisions have been made, namely, to include: (1) viruses whose longevity *in vitro* is less than 7 days and (2) those that can withstand aging for 7 days or more. Since the temperature at which the extracts are stored greatly influences the longevity of a virus, a standard temperature should be selected for this test. On the whole, room temperature (about 22° C.) may prove most suitable and convenient. While this temperature may be more difficult to maintain at a constant level in some laboratories than higher or lower temperatures, the majority of workers should find it the easiest to approximate without special equipment. The 7-day period for aging is suggested partly because it is a convenient length of time, and partly because it serves to separate, among others, viruses of the cucumber mosaic type from certain forms of greater longevity.

The critical thermal-death-point temperatures selected are those of 60° and 80° C. In the present key, a mechanically-transmitted virus will fall into one of several categories, according to its grouping in the preceding classes and whether or not its thermal death point is above or below either 60° or 80° C. (Table 1). Thus, unless the thermal death point of a virus lies close to the critical temperature, it may not be necessary to make a very accurate determination for purposes of identification. A reasonable degree of standardization of methods is essential in such tests of properties. Everything considered, it seems simplest in the longevity and thermal-death-point determinations to use undiluted extract or extract diluted only 1 to 1. Most of the thermal death points accepted for the present key are based upon 10-minute exposures of 1 or 2 cc. of plant extract in thin-walled test tubes. The host from which the virus is extracted does not appear markedly to influence the results secured, and, while in most cases it may be best to use the natural or most common host, in other cases the determination may be of greater value if made upon certain susceptible hosts that are known to be affected by other related viruses.

The criterion of transmission by grafting, used in one section of the key, is not always a simple one in practice, especially in the case of monocotyledons or herbaceous dicotyledons. For the present, however, it must remain an important diagnostic feature for certain viruses, even though negative results may be inconclusive, for the reason that transmission in this manner may constitute the only proof yet available of the virus nature of particular diseases to be included in this classification.

The diagnostic value of natural and differential hosts is obviously great in many instances. Nevertheless, it is clear from the narrow host ranges of some viruses and the wide and overlapping host ranges of others that, in general, only a limited relationship may be concluded to exist between plant viruses and the different plant families. Description of a virus on a single host only, or on a limited group of hosts, without regard to the value of these for differential purposes, has often led to synonomy or to confusion in nomenclature.

Although, as has been pointed out, symptom expression may be unreliable or of limited value for diagnostic purposes when the specific virus concerned is to be determined, it nevertheless seems desirable to include some reference to symptoms in the descriptions of all viruses. In the case of those transmitted only with difficulty or not at all by plant extract, symptom expression sometimes constitutes one of the chief diagnostic characters. The undesirable features of symptomatology for descriptive purposes relate largely to the use of and reliance upon minute details not in themselves sufficiently characteristic or distinctive to be of differential value. For present purposes, description of symptoms may be confined chiefly to such terms as mottling, chlorosis, malformation and necrosis, when other more fundamental characters of the virus also are given.

Other criteria may be useful on rare occasions for distinguishing particular forms, as, for instance, transmissibility through seed or through soil, and the presence of intracellular inclusions, or x-bodies. With the discovery of an increasing number of "strains" of certain viruses, still other diagnostic characters will no doubt need to be introduced, or present ones expanded, in order to meet future requirements.

The descriptive key here presented has been developed according to these considerations (Table 3). Actually, it has been possible to place about 50 forms in their respective positions in the system. This number, representing about one-third to one-half of the reported viruses, is believed to be sufficiently inclusive to illustrate most of the virus types or "type-forms." The omission of certain apparently well-described viruses is in many cases due to the lack of reliable information on one of the specific diagnostic features, as, for instance, the insect relations. Sufficient information is not yet available on a large percentage of plant viruses to allow them to be definitely placed in the key, and a considerable number must consequently remain in the undetermined group until the necessary information has been secured. While this group need not concern us greatly in the present connection, it has seemed worth while to list most of these viruses in such a way as to indicate some of the chief diagnostic features that are lacking (Table 2).

As an example of incomplete description may be cited the senior writer's

TABLE 2.—*A list of some incompletely described viruses, showing the chief undetermined or doubtful diagnostic features*

+ determined; ? undetermined or doubtful

Transmission by			Properties	Virus of
Sucking insects	Plant extract	Grafting		
+	+		?	clover mosaic crucifer mosaic Easter-lily mosaic iris mosaic lettuce mosaic potato internal-parenchyma necrosis potato spindle tuber potato unmottled curly dwarf sugar-cane mosaic tulip breaking
?	+		+	dock mosaic tobacco bleaching mosaic tobacco mild mosaic tobacco speckled mosaic tomato stem necrosis
?	+		?	anthurium mosaic cowpea mosaic delphinium mosaic grape mosaic hop chlorosis lily-of-the-valley mosaic narcissus gray disease petunia mosaic potato aucuba mosaic potato calico potato paracrinkle rose wilt soy-bean mosaic sweet-potato mosaic tobacco etch tobacco Rotterdam-B disease tomato bunchy top vegetable-marrow mosaic wheat rosette white-clover mosaic
?	?	+		apple mosaic black-currant reversion black-locust brooming disease cherry buckskin citrus chlorosis dahlia ring spot fig mosaic hop mosaic hop nettlehead peach little peach peach mosaic peach phony disease peach red suture pelargonium mosaic

TABLE 2.—(*Continued*)

Transmission by			Properties	Virus of
Sucking insects	Plant extract	Grafting		
?	?	+		plum pox potato apical leaf roll potato interveinal mosaic potato supermild mosaic potato yellow top rose chlorosis spindle-tree chlorosis tomato big bud tomato witches' broom vine court-noué
?	?	?		anemone alloiophylly bean rugose mosaic dahlia oakleaf gladiolus mosaic hippeastrum mosaic potato giant hill raspberry streak rubber brown bast sweet-potato mosaic violet mosaic

account of several viruses on tobacco (4), which disregards possible insect relations. Similarly, E. M. Johnson's description of virus diseases of tobacco in Kentucky (3) omits certain essentials for purposes of adequate description and identification. It is in such instances, where transmissibility by plant extract is known but property determinations are lacking, or where transmissibility by grafting is determined but insect relations uncertain, that the largest groups of imperfectly described viruses fall. It is hoped that, as virologists find opportunity to work with viruses whose descriptions are yet incomplete, the necessary effort to obtain the desired information will be made.

As an illustration of what may be considered a sufficiently complete description of a virus transmissible mechanically by plant extract, the following example may be given:

Ordinary tobacco mosaic virus (*tobacco virus* 1). Type: Allard, H. A., Jour. Agr. Research **6**: 649–674. 1916. Transmissible with difficulty by aphids (*Myzus pseudosolani* Theob. and others) from tomato (*Lycopersicon esculentum* Mill.), but not from tobacco. Readily transmitted mechanically by plant extract. Longevity *in vitro*, 3 months or more. Thermal death point 92° C., 10 minutes. Tobacco (*Nicotiana tabacum* L.) and many other solanaceous and non-solanaceous plants susceptible. Necrotic local lesions on *Nicotiana glutinosa* L. Inclusion bodies (x-bodies and striated crystals) present in tobacco and many other hosts. Varying shades

of green coarse mottling on tobacco. Attenuated strains and other strains, separable chiefly on basis of symptoms, occur in nature. Distribution coexistent with tobacco culture.

DISCUSSION

The placing of the viruses in the key has been influenced to some extent by individual judgment as to what may or may not be accepted as adequate determination of any particular character. The element of error in the position assigned to some forms may, therefore, be considerable, and we may reasonably expect to find certain viruses needing to be shifted from time to time, following further investigations.

As far as is known, no previous serious attempt has been made to bring all known forms of plant virus diseases together in related groups, although several suggested classifications have been made for such groups of virus diseases as those of the potato (2, 7), and of tobacco (3). The greatest weakness of these systems has been the lack of applicability of the principles involved to larger groups of known virus diseases. Such broader groupings, based upon symptom expression, as "yellows" and "mosaic," are also no longer satisfactory as a starting point for differentiation.

It may be argued that in the absence of any morphological grounds differentiation and description of the virus itself is impractical. Considered from a broad point of view, the progress made in the recognition of such invisible entities as atoms, vitamins, and genes should be encouraging. More specifically, however, in the case of the viruses that are readily transmitted by plant extract, we have means of description that are quite as reliable and satisfactory as those available in the case of many morphologically related forms of bacteria and fungi, where behavior and reaction to specific conditions are basic to differentiation. In the case of viruses not transmissible by plant extract, this characteristic itself serves in part as an advantage in that it excludes a large group which might otherwise need to be separated by more artificial and complicated methods. Distinctions in this latter group, fortunately, may usually be accomplished by means of insect vectors.

One of the most promising fields for research on plant viruses relates to the study of the similarities and relationships between various viruses and groups of viruses. In the earlier period of research, the similarity of different forms was stressed; more recently their dissimilarity has been emphasized. The present period of investigation is already tending strongly toward the recognition of different "strains" or forms of one virus, closely related in their more fundamental characters, but differing in minor features or in symptom expression. Hence, virus research has already reached the stage where studies on a particular virus that disregard

TABLE 3.—*A descriptive key for plant viruses*

A_1. Transmissible by sucking insects.

B_1. NOT TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.

C_1. Not transmissible by aphids.

D_1. Transmissible by leaf hoppers.

E_1. China aster (*Callistephus chinensis* Nees) susceptible.
Vein clearing; chlorosis of leaves and flowers; proliferation.
Transmitted by *Cicadula sexnotata* Fall.
ASTER YELLOWS VIRUS

E_2. Cranberry (*Vaccinium macrocarpon* Ait.) susceptible.
Malformation of flowers; proliferation of lateral buds.
Transmitted by *Euscelis striatulus* Fall.
CRANBERRY FALSE-BLOSSOM VIRUS

E_3. Peach (*Prunus persica* Sieb.) susceptible.
Chlorosis and necrosis of foliage; proliferation of lateral buds.
Transmitted by *Macropsis trimaculata* Fitch.
PEACH YELLOWS VIRUS

E_4. Sandal (*Santalum album* L.) susceptible.
Chlorosis, proliferation and necrosis; malformation of floral parts.
Transmitted by *Moonia albimaculata* Distant.
SANDAL SPIKE VIRUS

E_5. Sugar beet (*Beta vulgaris* L.) susceptible.
Vein clearing, leaf rolling, vein enations, phloem necrosis.
Transmitted by *Eutettix tenella* Baker.
SUGAR BEET CURLY TOP VIRUS

E_6. Corn (*Zea mays* L.) susceptible.

F_1. Inclusion bodies present.
Mottling along veins.
Transmitted by *Peregrinus maidis* Ashm.
CORN MOSAIC VIRUS

F_2. No inclusion bodies present.
Mottling along veins.
Transmitted by *Cicadulina mbila* Naude.
CORN STREAK VIRUS

E_7. Sugar cane (*Saccharum officinarum* L.) susceptible.
Galls on leaves and stalks.
Inclusion bodies present.
Transmitted by *Perkinsiella vastatrix* Breddin and *P. saccharicida* Kirk.
SUGAR-CANE FIJI-DISEASE VIRUS

E_8. Rice (*Oryza sativa* L.) susceptible.
Mottling and stunting.
Inclusion bodies present.
Transmitted by *Nephotettix apicalis* Motsch.
RICE STUNT-DISEASE VIRUS

D_2. Not transmissible by leaf hoppers.

E_1. Transmissible by thrips.
Pineapple (*Ananas sativus* L.) susceptible.
Chlorotic spots and concentric rings; necrosis.
Transmitted by *Thrips tabaci* Lind.
PINEAPPLE YELLOW-SPOT VIRUS

E_2. Not transmissible by thrips.

F_1. Transmissible by white fly.

G_1. Tobacco (*Nicotiana tabacum* L.) susceptible.
Vein thickening and enations; leaf curling.
Transmitted by *Bemisia* sp.
TOBACCO LEAF-CURL VIRUS

G_2. Cotton (*Gossypium herbaceum* L.) susceptible.
Vein thickening and enations; leaf curling.
Transmitted by *Bemisia gossypiperda* Misra & Lamba.
COTTON LEAF-CURL VIRUS

F_2. Not transmissible by white fly.

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TABLE 3.—(*Continued*)

C_2. Transmissible by aphids.
D_1. Rosaceae susceptible.
E_1. Raspberry (*Rubus strigosus* Michx.) susceptible.
F_1. Transmitted by *Amphorophora rubi* Kalt.
G_1. Mottling, necrosis, and malformation.
RED RASPBERRY MOSAIC VIRUS
G_2. Yellow mottling, chlorosis, necrosis, and malformation.
RASPBERRY YELLOW MOSAIC VIRUS
F_2. Transmitted by *Aphis rubiphila* Patch.
Leaves dark green, sometimes bronzed, curled.
G_1. Black raspberry not susceptible.
RASPBERRY LEAF-CURL VIRUS (ALPHA TYPE)
G_2. Black raspberry susceptible.
RASPBERRY LEAF-CURL VIRUS (BETA TYPE)
E_2. Blackberry (*Rubus* spp.) susceptible.
Mild mottling and dwarfing.
Transmitted by *Capitophorus tetrarhodus* Walker.
BLACKBERRY DWARF VIRUS
E_3. Strawberry (*Fragaria* spp.) susceptible.
Transmitted by *Myzus fragaefolii* Ckll.
F_1. Chlorosis and malformation; no proliferation.
STRAWBERRY XANTHOSIS VIRUS
F_2. Numerous spindling petioles.
STRAWBERRY WITCHES'-BROOM VIRUS
E_4. Ocean spray (*Holodiscus discolor* Max.) susceptible.
Brooming and dwarfing.
Transmitted by *Aphis spiraecae* Schout.
OCEAN-SPRAY WITCHES'-BROOM VIRUS
D_2. Rosaceae not susceptible so far as known.
E_1. Potato (*Solanum tuberosum* L.) susceptible.
Transmitted by *Myzus persicae* Sulz.
F_1. Leaf rolling, chlorosis, brittleness, phloem necrosis.
POTATO LEAF-ROLL VIRUS
F_2. Chlorosis; necrosis of pith in stem and tuber.
POTATO YELLOW-DWARF VIRUS[a]
E_2. Dahlia (*Dahlia variabilis* Desf.) susceptible.
Mottling, malformation, often vein necrosis.
Inclusion bodies present.
Transmitted by *Myzus persicae* Sulz.
DAHLIA MOSAIC VIRUS
E_3. Banana (*Musa cavendishii* Lamb.) susceptible.
Mottling, malformation, and necrosis.
Transmitted by *Pentalonia nigronervosa* Coq.
BANANA BUNCHY-TOP VIRUS
E_4. Peanut (*Arachis hypogaea* L.) susceptible.
Chlorosis, stunting, and mottling.
Transmitted by *Aphis leguminosae* Theob.
PEANUT ROSETTE VIRUS
E_5. Alfalfa (*Medicago sativa* L.) susceptible.
Mottling, sometimes ring-like.
Transmitted by *Illinoia* (*Macrosiphum*) *pisi* (Kalt.) Baker.
ALFALFA MOSAIC VIRUS
E_6. Garden pea (*Pisum sativum* L.) susceptible.
Mottling and malformation.
Transmitted by *Illinoia* (*Macrosiphum*) *pisi* (Kalt.) Baker.
PEA MOSAIC VIRUS
E_7. Easter lily (*Lilium longiflorum* Baker) susceptible.
Chlorosis and downward curling of leaves.
Transmitted by *Aphis gossypii* Glov.
EASTER-LILY YELLOW FLAT VIRUS

[a] Also reported as transmitted by a leaf hopper (*Agallia sanguinolenta* Prov.).

TABLE 3.—(*Continued*)

B$_1$. TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.
C$_1$. Longevity *in vitro* less than 7 days at 22° C.
D$_1$. Thermal death point below 60° C. (10 minutes).
E$_1$. Not transmissible by aphids.
F$_1$. Transmissible by thrips.
Transmitted by *Frankliniella insularis* Franklin and *Thrips tabaci* Lind.
Tomato (*Lycopersicon esculentum* Mill.) susceptible.
Bronzing, ring chlorosis, and necrosis.
TOMATO SPOTTED WILT VIRUS

E$_2$. Transmissible by aphids.
F$_1$. Tobacco (*Nicotiana tabacum* L.) not susceptible.
G$_1$. Potato (*Solanum tuberosum* L.) susceptible.
H$_1$. Mild mottling, slight wrinkling.
POTATO MILD MOSAIC VIRUS
H$_2$. Pronounced mottling and wrinkling.
POTATO CRINKLE MOSAIC VIRUS
G$_2$. Potato (*S. tuberosum* L.) not susceptible.
Bean (*Phaseolus vulgaris* L.) susceptible.
H$_1$. Transmissible through seed.
Mottling and malformation.
BEAN MOSAIC VIRUS
H$_2$. Not transmissible through seed.
Intense yellow mottling and malformation.
BEAN YELLOW MOSAIC VIRUS
F$_2$. Tobacco (*N. tabacum* L.) susceptible.
Sugar beet (*Beta vulgaris* L.) susceptible.
Mottling and malformation.
SUGAR-BEET MOSAIC VIRUS

D$_2$. Thermal death point 60° C. or above (10 minutes).
E$_1$. Transmissible by aphids.
F$_1$. Celery (*Apium graveolens* L.) susceptible.
G$_1$. Watermelon (*Citrullus vulgaris* Schrad.) susceptible.
Mottling and necrosis on celery.
CELERY MOSAIC VIRUS
G$_2$. Watermelon not susceptible.
Mottling, necrosis, and malformation on cucumber (*Cucumis sativus* L.)
CUCUMBER MOSAIC VIRUS
F$_2$. Potato (*Solanum tuberosum* L.) susceptible.
G$_1$. Tobacco (*Nicotiana tabacum* L.) susceptible.
Mild veinbanding symptoms on tobacco.
POTATO VEINBANDING VIRUS
G$_2$. Tobacco (*N. tabacum* L.) not susceptible.
Mottling; rolling of upper leaves on potato.
POTATO LEAF ROLLING MOSAIC VIRUS
F$_3$. Onion (*Allium cepa* L.) susceptible.
Chlorosis and malformation.
ONION YELLOW DWARF VIRUS
E$_2$. Not transmissible by aphids.

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C$_2$. Longevity *in vitro* 7 days or more at 22° C.
D$_1$. Thermal death point below 80° C. (10 minutes).
E$_1$. Transmissible by aphids.
Alfalfa (*Medicago sativa* L.) susceptible.
Mottling on alfalfa; local necrosis on bean (*Phaseolus vulgaris* L.)
ALFALFA VIRUS 2
E$_2$. Not transmissible by aphids.

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D$_2$. Thermal death point 80° C. or above.

TABLE 3.—(*Continued*)

E_1. Transmissible by aphids from tomato (*Lycopersicon esculentum* Mill.).
Tobacco (*N. tabacum* L.) susceptible.
Local necrosis on *Nicotiana glutinosa* L.
Inclusion bodies present.
F_1. Green coarse mottling pronounced on tobacco.
ORDINARY TOBACCO MOSAIC VIRUS
F_2. Mild or no mottling on tobacco.
ATTENUATED TOBACCO MOSAIC VIRUS
F_3. Yellow mottling on tobacco.
YELLOW TOBACCO MOSAIC VIRUS
E_2. Not transmissible by aphids from tomato.

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A₂. Not transmissible by sucking insects.
B_1. TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.
C_1. Longevity *in vitro* 7 days or more at 22° C.
D_1. Thermal death point 60° C. or above (10 minutes).
Tobacco (*N. tabacum* L.) susceptible.
E_1. Tomato (*Lycopersicon esculentum* Mill.) susceptible.
F_1. Faint or mild mottling on tobacco.
POTATO MOTTLE VIRUS
F_2. Small necrotic rings on tobacco.
POTATO RING SPOT VIRUS
E_2. Tomato (*L. esculentum* Mill.) not susceptible (*i.e.*, to juice inoculation).
Large necrotic rings on tobacco (*N. tabacum* L.).
TOBACCO RING SPOT VIRUS
D_2. Thermal death point less than 60° C. (10 minutes).

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C_2. Longevity *in vitro* less than 7 days at 22° C.

* * * * * *

B_2. NOT TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.
C_1. Transmissible by grafting.
D_1. Potato (*Solanum tuberosum* L.) susceptible.
E_1. Yellow blotches on leaves.
POTATO AUCUBA MOSAIC VIRUS
E_2. Brooming; dwarfing of leaves and tubers.
POTATO WITCHES'-BROOM VIRUS
D_2. Potato (*S. tuberosum* L.) not susceptible.
E_1. Abutilon (*Abutilon theophrasti* Medic.) susceptible.
Mottling or variegation of leaves.
ABUTILON MOSAIC VIRUS
E_2. Peach (*Prunus persica* Sieb.) susceptible
Chlorosis and rosetting of foliage.
PEACH ROSETTE VIRUS
C_2. Not transmissible by grafting so far as known.
D_1. Transmissible by other vegetative means.
E_1. Potato (*Solanum tuberosum* L.) susceptible.
Gigantism of vines and tubers, no mottling.
POTATO GIANT HILL VIRUS
E_2. Raspberry (*Rubus strigosus* Michx.) susceptible.
Leaf curling and blue stripes on canes.
RASPBERRY STREAK VIRUS
D_2. Not transmissible by other vegetative means.
Not transmissible by seed.
Not transmissible through soil.
Cotton (*Gossypium herbaceum* L.) susceptible.
Malformation, sterility, no mottling.
COTTON CRAZY TOP VIRUS

the probable relationships of other strains or forms are handicapped at the outset. Although a fairly clear conception of many groups of viruses has now come to be formed, the relation of these groups to one another has not yet been given much consideration. With the wealth of material available, investigations aimed primarily at studying such relationships are likely to yield some most interesting results of taxonomic value. While it may yet be too early to attempt a sound classification scheme for plant viruses based upon "natural" relationships, the possibilities in this direction are perhaps greater than is generally supposed. Viruses already recognized perhaps represent the extremes of a variety of types between which may be found a remarkable gradation of forms. The ordinary tobacco-mosaic virus, for instance, may be taken as a "mutating" form. With respect to symptom expression, it is known to "mutate" in nature and it may be altered in certain respects by artificial means. It is, or it is not, transmitted by aphids according to the host used as a source of the virus. Yet, as far as its properties are concerned, the ordinary tobacco-mosaic virus is remarkably stable, and the stability of the various "strains" of this virus also is well established. Compared to other known viruses, these and other characteristics suggest that the ordinary tobacco-mosaic virus is probably an intermediate form, and does not represent a natural extreme. It may be futile to speculate as to which forms represent the extremes of a theoretical evolutionary scale, but without attempting to indicate which might be regarded as the highest and most specialized type and which the lowest or most primitive, it is at least suggestive to place the aster-yellows virus at one end of the scale and, at the other end, a virus that may eventually be shown to be transmissible only through vegetative means. Or, there is some reason to believe that a virus that is least specialized, *i.e.*, transmitted by various means and to a wide host range, may more truly represent one end of such a scale. As far as our own present conception of the grouping and order of classification of plant viruses is concerned, the main features are represented in the descriptive key presented.

SUMMARY

The need for a more complete and systematic description of plant viruses is discussed. The suggestion is again made that description should be based upon the characters of the virus itself rather than upon the disease which it causes.

The chief diagnostic features of a virus that are of value for descriptive purposes are believed to be: (1) Modes of transmission; (2) natural or differential hosts; (3) longevity *in vitro;* (4) thermal death point; (5) certain distinctive or specific symptoms.

Transmission by sucking insects and the identity of the vector concerned,

together with mechanical transmission by plant extract and transmission by grafting, constitute the main bases for differentiation.

By utilizing the above criteria in particular, it has been possible to draw up a descriptive key for plant viruses that places about 50 of the known forms in several fairly well-defined groups.

The suggestion is made that the various groups bear a certain natural relation to each other, and that a logical basis for classification may develop for more intensive studies of these relationships.

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A LEAF SPOT OF BENT GRASSES CAUSED BY HELMINTHOSPORIUM ERYTHROSPILUM, N. SP.

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HISTOLOGICAL REVIEW, DISTRIBUTION, HOST RANGE, ECONOMIC IMPORTANCE

In a comparative account (1, p. 684) of various graminicolous members of the genus Helminthosporium that was published in 1923, reference was made to the occurrence on redtop, *Agrostis alba* L., and on thin grass, *A. perennans* (Walt.) Tuckerm., of 2 apparently undescribed species of Helminthosporium having larger conidia than *H. dematioideum* Bub. & Wrob., a form now and then also found on these grasses. The 2 fungi in question had been observed frequently on withered leaves of the bent grasses collected on Long Island near Brooklyn, N. Y., as well as in southwestern Connecticut between Stamford and Norwalk at different times late in June and during July in both 1920 and 1921. As in the absence of distinctive lesions the withering had much the appearance of drought injury, it was not possible then to attribute to either of the fungi any definitely disease-producing rôle.

Beginning with August, 1921, observations on redtop and other bent grasses were continued in the District of Columbia and adjacent portions of Maryland and Virginia. A withering of foliage resembling that previously seen in New York and Connecticut, was in evidence here also, and mostly, again, without any intervention of well-marked lesions. Microscopic examination of the dead leaves rather regularly revealed the presence of conidiophores and conidia of the one of the two larger species of Helminthosporium that had been by far the more abundant on redtop in the region surrounding New York City. During July, 1923, this species occurred on redtop in such definite relation to dull reddish grey foliar lesions that its causal connection with the injury became entirely obvious. In a brief abstract (3) based on observations then made the conidia were described as straight, cylindrical, olivaceous, 1- to 9-times septate, and germinating by the production of lateral and oblique germ tubes from intermediate as well as end segments. The details given were thus sufficient to place the fungus in the series of forms having their ascigerous stages in the genus Pyrenophora, especially as the antithesis between the two main series of graminicolous species of Helminthosporium with respect to both the conidial and the sexual stages was pointed out in another abstract (2) that appeared at the same time. Mention was made of the production of numerous small sclerotia in culture as a feature distinguishing the fungus from the parasite

causing leaf spot of oats; and the smaller dimensions of its conidia were cited as distinguishing it from *H. siccans* Drechsl., likewise a member of the Pyrenophora series.

Once the appearance of the more distinctive type of foliar injury due to the parasite was known, it was not difficult to recognize the pathological effects of the fungus on redtop in stands near Washington, D. C., nearly every season following 1923. When occasion offered opportunity for observation elsewhere, the same injury in association with the same fungus was found in various localities in the Middle West; as, for example, near East Lansing, Mich., in July, 1927; near La Fayette, Ind., in August, 1928; near Madison, Wis., in July, 1931; and near Columbus, Ohio, in July, 1933. Apparently the parasite is widely distributed wherever redtop is grown in the more humid States of our northern and middle latitudes, its prevalence and abundance here in any locality and season depending much on local weather conditions. All in all it seems certainly to be one of the most serious parasites affecting redtop, not any of which, to be sure, are especially devastating in their action. The economic loss accruing from it is perhaps not very considerable. Yet the possibility is hardly to be overlooked that damage to pasture and forage grasses brought about by leaf diseases may be greater than, in the absence of any good means of estimating injury of such origin, is now generally supposed.

Besides the agriculturally very valuable redtop, the fungus under discussion is known to attack also seaside bent, *Agrostis palustris* Huds., (formerly cited as *A. maritima* Lam.), and colonial bent, *A. tenuis* Sibth. Colonial bent was seen attacked only feebly, the strain of this grass growing at Arlington Experiment Farm, Rosslyn, Va., in 1928, showing only a few scattered lesions (Fig. 1, F–H) during the prolonged wet period in September of that year. The remarkably heavy infection that took place on seaside bent at that time was referred to in an earlier paper (4, pp. 129, 130, 132). Several of the strains of creeping bent, *A. palustris* Huds., (previously cited as *A. stolonifera* L.), widely used for turf on golf courses, bore lesions that appeared very probably referable to the Helminthosporium common on redtop, though the abundance of the zonate-eyespot fungus, similarly encouraged by the wet weather, precluded a wholly certain identification. Even greater difficulty attended the attempted identification of reddish lesions found on velvet bent, *A. canina* L., owing to the narrowness of the leaves taken together with the abundance of zonate-eyespot lesions in all stages of development.

SYMPTOMS AND PATHOLOGICAL EFFECTS

That the parasite has so long escaped observation in spite of its widespread distribution on an important economic plant, is undoubtedly to be

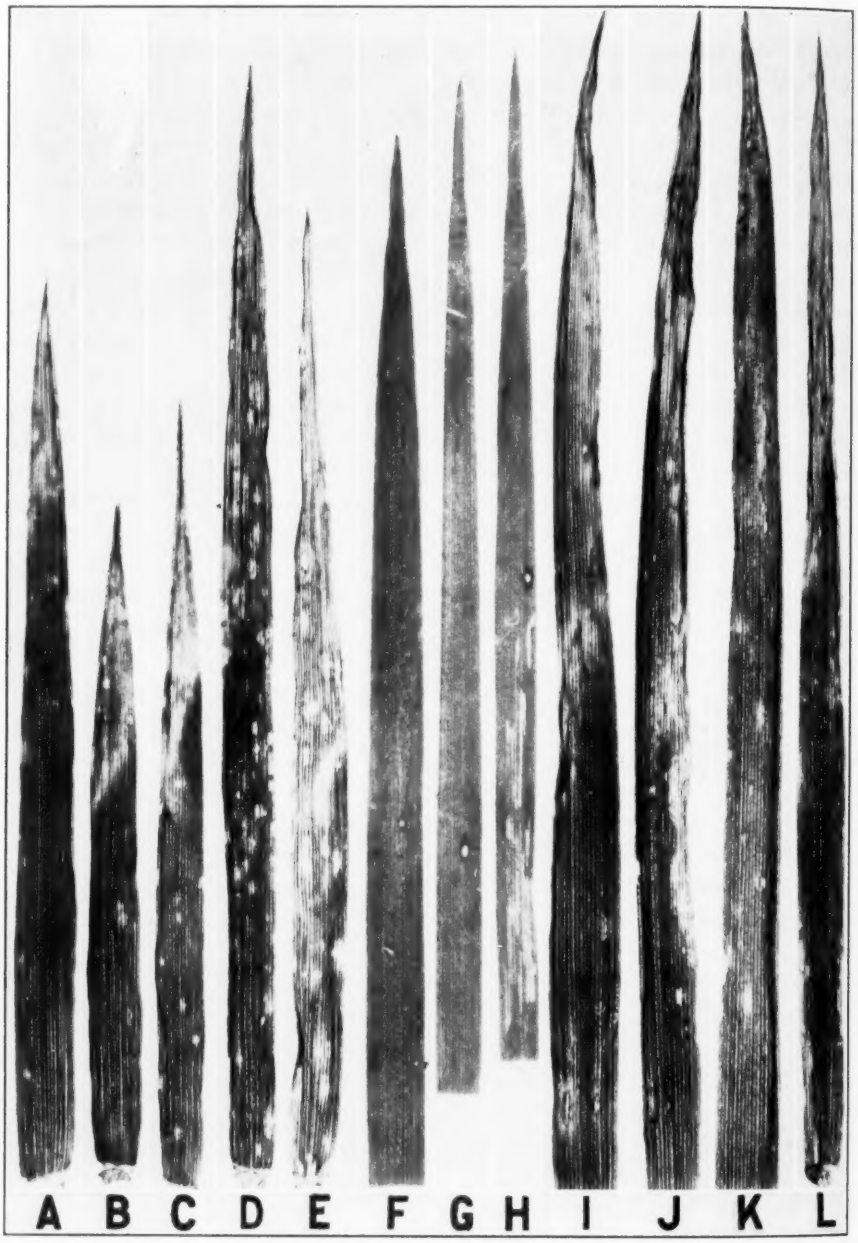

FIG. 1. A–E. Redtop leaves with lesions and general withering effects brought about by *Helminthosporium triseptatum;* photographed from material collected at Arlington Experiment Farm, Sept. 11, 1926. ×2. F–H. Leaves of colonial bent with single lesions due to invasion by *H. erythrospilum;* photographed from material collected at Arlington Experiment Farm, Sept. 26, 1928. ×2. I–L. Leaves of seaside bent collected at Arlington Experiment Farm, Sept. 28, 1928, showing definite lesions due to *H. erythrospilum* that had developed in rainy weather, and withering effects that soon appeared when drier conditions later intervened. ×2.

attributed in large part to the frequently deceptive appearance of the injury caused by it. In dry seasons and often even in moderately wet seasons the local discoloration associated with the inception and enlargement of separate invaded areas soon becomes obscured in the general withering of the leaf that follows apparently as a secondary effect of invasion. Consequently, affected redtop leaves frequently present a drought-stricken appearance, though the moisture available may be more than enough to keep healthy plants in full vegetative luxuriance.

During the 15 seasons in which my observations were continued, the essentially local character of the separate infections was never brought into relief more clearly than in the heavy attack of the fungus on seaside bent in September, 1928, to which reference has already been made. With the unusually moist and warm weather that came in the first 3 weeks of that month, infections multiplied on the luxuriant foliage, while at the same time the secondary withering effects were almost entirely avoided (Fig. 2, A–L). On most of the leaves were borne lesions from 5 to 25 in number, individually appearing often as straw-color spots, 0.5 to 0.8 mm. wide and 0.5 to 2.5 mm. long, surrounded by a reddish or brownish red border of variable width. Sometimes the straw-color center was very minute or entirely absent, and the spot then rather closely resembled the lesions that had become familiar on redtop under more ordinary weather conditions. Collections made during the prolonged intervals of precipitation showed a large proportion of the discolored spots surrounded individually by a water-soaked belt of variable width, representing undoubtedly an increment of newly occupied tissue. These zonal increments are illustrated fairly well in figure 2, D–L, showing affected leaves photographed immediately on collection after 16 hours of drizzling rain. The more extensive zonate lesions (Fig. 2, K, L) thus sometimes came to present a pattern somewhat suggestive of lesions caused by *Helminthosporium giganteum* on its more favorable hosts, among which, however, the strain of seaside bent in question is not to be reckoned. The differences between the simple eyespot lesions on this grass caused by *H. giganteum* and those due to the fungus under discussion have been previously set forth (4, p. 132), and become readily evident on a comparison of the pertinent illustrations of the earlier paper (4, Pl. 1, J–S) with those included in figure 2 of the present contribution. Rather curiously, the lesions produced by *H. giganteum* on seaside bent resemble those produced by the leaf-spot fungus on the same host (Fig. 2, A–L) much less than they resemble those produced by the latter parasite on the specifically different colonial bent (Fig. 1, F–H).

Weather conditions that permit the development of leafspot lesions as definite and numerous as those present on the leaves shown in figure 2,

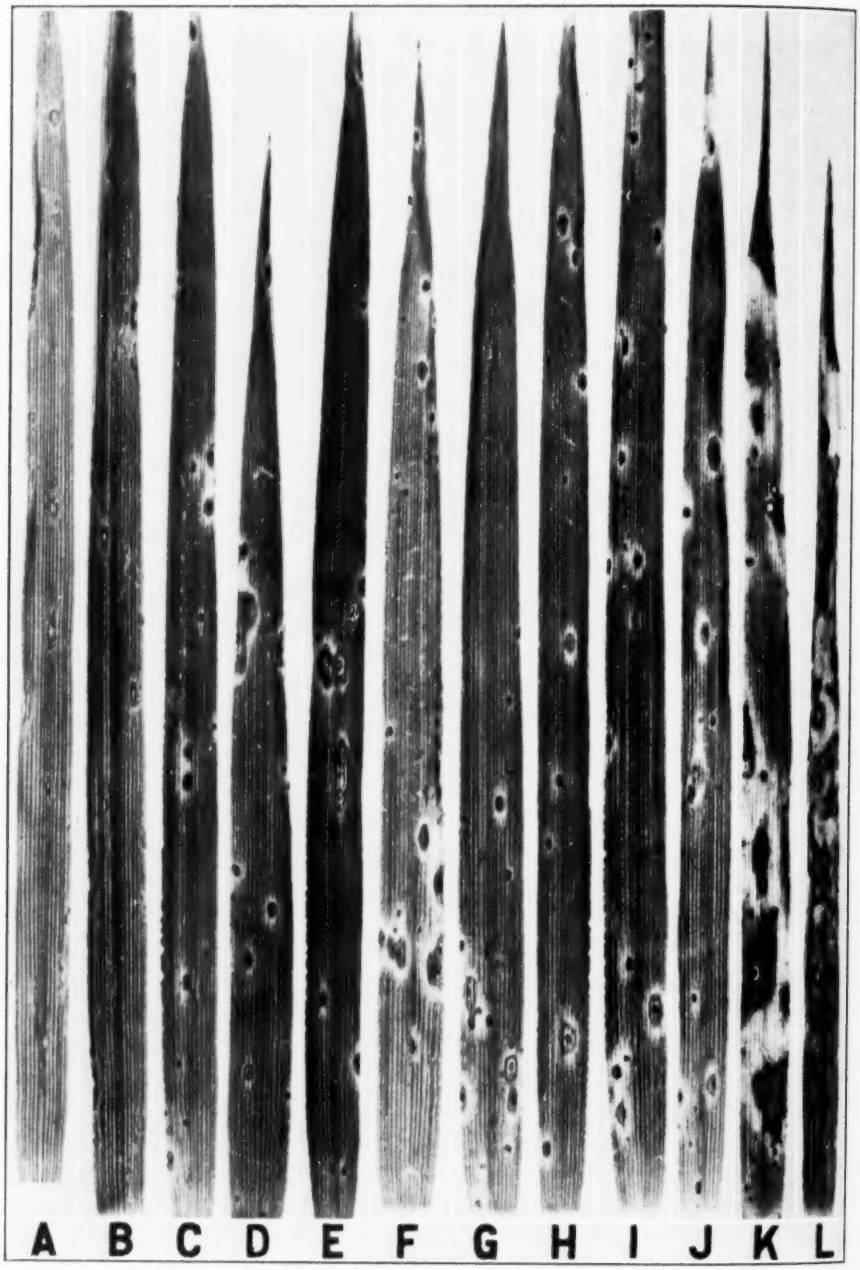

FIG. 2. A–L. Leaves of seaside bent arranged approximately in the order of increasing severity of attack by *Helminthosporium erythrospilum*, collected at Arlington Experiment Farm, Sept. 19, 1928, after 16 hours of misty rain. The light zones surrounding many of the spots represent water-soaked areas newly invaded by the fungus. Symptoms of withering are absent except in K, L. ×2.

are not wont to occur frequently, at least in the regions in which my observations were made. When such conditions come to an end, withering of the more seriously affected leaves soon intervenes; and with the consequent obliteration, in large part, of local discoloration, much of the distinctiveness of the earlier pathological picture is lost. Thus several days after the rains prevailing when the material shown in figure 2 was collected had come to an end, the diseased foliage in the same plot of seaside bent from which the collections were made showed the leafspot discoloration considerably faded, and the blades themselves in process of withering from tip toward base (Fig. 1, I–L).

COMPARISON WITH A REDTOP LEAF SPOT DUE TO HELMINTHOSPORIUM TRISEPTATUM

In the region surrounding New York City, as has been noted previously, *Helminthosporium dematioideum* was occasionally found during the season of 1920 on withered foliage of redtop and of thin grass. However, no well marked foliar lesions could at any time be connected with the presence of this fungus, even though it was encountered on redtop in small quantity from time to time in later seasons near the District of Columbia. Manifestly not much pathogenic activity is to be attributed to *H. dematioideum* as far as bent grasses are concerned. Indeed it would not be difficult to deny any injurious action on these grasses whatsoever, except for the fact that the foliage of sweet vernal grass, *Anthoxanthum odoratum* L., is almost equally free of local discoloration up to the time it begins to shrivel and put forth conidial apparatus of *H. dematioideum* in such quantity and with such regularity as to indicate a parasitic relationship.

In several of the last 14 seasons *Helminthosporium triseptatum* Drechsl., a species first described (1, pp. 685–686) as occurring on velvet grass, *Holcus lanatus* L., has been found also on fading leaves of redtop as well as of timothy, *Phleum pratense* L., in Virginia and Maryland near Washington, D. C. In some especially favorable redtop material this fungus could be made out as the cause of rather minute foliar lesions consisting of a pale central area surrounded by a salmon-color zone (Fig. 1, A–E). Horsfall (5) found a fungus he identified provisionally as *H. triseptatum* to be the cause of a leafspot on redtop and on orchard grass, *Dactylis glomerata* L., in the State of New York. The lesions on redtop described by Horsfall would seem to have been somewhat darker than those caused by *H. triseptatum* on this grass in Virginia. In New York, moreover, the spots were described as becoming rapidly elongated longitudinally, forming streaks through which the death of the leaf is brought about; whereas, in Virginia, such death would seem to be introduced by general withering of the organ as a whole. These differences very probably find their explanation in the

rather considerable difference in environmental conditions obtaining between the two regions. Whatever the explanation may be, however, the individual lesions due to *H. triseptatum* in Virginia and Maryland, are usually distinguishable from those due to the fungus herein described, in their smaller dimensions and paler coloration. Of the two parasites, the former appears to be by far the less destructive one, at least as far as the bent grasses are concerned.

MORPHOLOGY OF THE CAUSAL FUNGUS ON THE NATURAL SUBSTRATA

The fungus responsible for the more serious, russet leaf spot is regularly to be found on the more extensive tracts of tissue killed by it (Fig. 3, A, a–c). Its conidiophores first generally appear in the centers of the larger discolored areas. Later they arise, somewhat more sparsely, from areas not included in the originally infected lesions. In either position they emerge from the host epidermis singly (Fig. 3, B, a–c, f) or in pairs (Fig. 3, B, d, e), rarely in larger groups. Emergence ordinarily takes place between epidermal cells; when stomata are utilized (Fig. 3, B, d, e) it is apparently more through accident than from physical constraint such as is evidently exerted by the structurally much sturdier epidermis of maize, *Zea mays* L., on the conidiophores of *H. turcicum* Pass. In dimensions, coloration, and septation, as in the presence of geniculate bends marking the points at which successive conidia were formed, the conidiophores of the present fungus show little to distinguish them from the homologous structures of congeneric parasites.

The cylindrical shape of the conidia (Fig. 3, C, a–z, aa–ad) and their indiscriminate mode of germination (Fig. 3, D, a–g) refers the fungus to the series of forms in Helminthosporium that have their ascigerous connections, wherever these are known, in the genus Pyrenophora—the series essentially corresponding, therefore, to the subgenus Cylindro-Helminthosporium of Nisikado (7, 8) and to the genus Drechslera of Ito (6). Though the conidia are by no means small in comparison with fungus spores generally, they are yet so much smaller than those of some of the larger members of the Pyrenophora series—*H. bromi* Died., *H. tritici-repentis* Died., and *H. teres* Sacc.—that the possibility of specific identity is convincingly excluded through the disparity in conidial size alone. Through their greater dimensions can be excluded from consideration also *H. tritici-vulgaris*, a species parasitic on wheat in Japan (7, 8), and *H. giganteum*, which, in view of its peculiarities in germination and in Hormodendron-like aerial proliferation, can only very doubtfully be considered a member of the series.

However, the differences in conidial dimensions are less pronounced when comparison is made with the less robust members of the Pyrenophora series. Among these members are to be reckoned, besides the barley-stripe

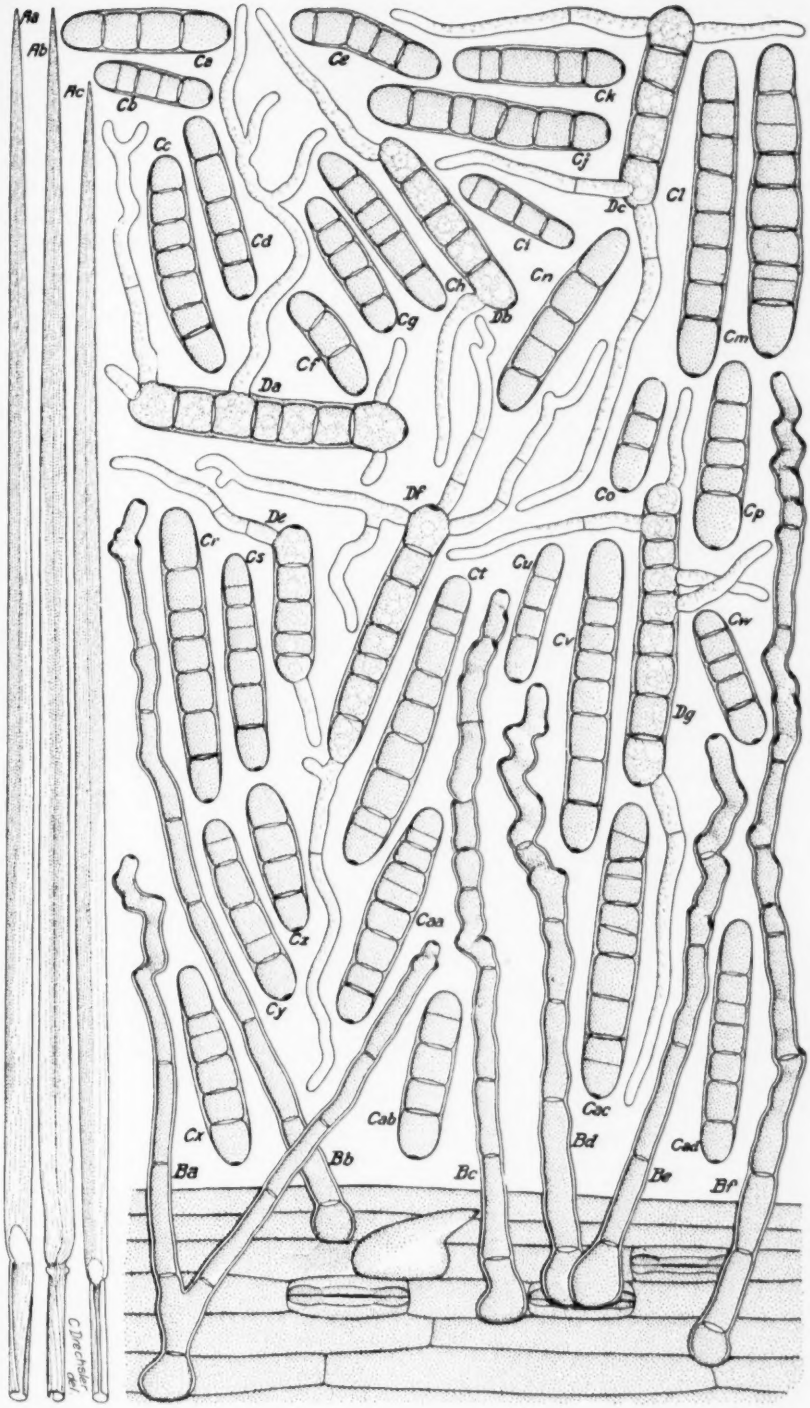

FIG. 3. *Helminthosporium erythrospilum* on redtop, drawn from material collected at Arlington Experiment Farm, July 16, 1923. A, a–c. Affected leaves of redtop, the more heavily stippled areas representing withered regions over parts of which conidiophores of the fungus are borne. ×1. B, a–f. Conidiophores emerging in various relationships to epidermal cells. ×500. C, a–z, aa–ad. Conidia from redtop leaves showing variations in size, shape, and septation. ×500. D, a–g. Conidia in early stages of germination. ×500.

TABLE 1.—*Lengths of 200 conidia produced by several species of Helminthosporium on their respective grass hosts in nature, distributed in classes with ranges of 10μ*

Fungus	21 to 30μ	31 to 40μ	41 to 50μ	51 to 60μ	61 to 70μ	71 to 80μ	81 to 90μ	91 to 100μ	101 to 110μ	111 to 120μ	121 to 130μ	131 to 140μ	141 to 150μ	151 to 160μ	161 to 170μ	Average length
H. erythrospilum (a)	2	15	29	29	48	40	24	12	1							64.3μ
H. erythrospilum (b)	1	16	18	43	42	48	23	9								65.2μ
H. dictyoides	2	14	20	23	24	29	38	30	13	4	2				1	74.5μ
H. siccans		4	13	19	20	49	41	26	16	5	3	3		1		80.1μ
H. gramineum		11	21	25	44	42	38	15	2	1		1				69.8μ
H. avenae		1	3	11	17	44	42	36	17	19	7	2	1			87.6μ

TABLE 2.—*Widths of 200 conidia produced by several species of Helminthosporium on their respective grass hosts in nature, distributed in classes with ranges of 1μ*

Fungus	8μ	9μ	10μ	11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	24μ	Average width
H. erythrospilum (a)	2	15	15	40	68	35	20	5									11.8μ
H. erythrospilum (b)	3	4	6	16	45	64	35	21	6								12.8μ
H. dictyoides				1		3	21	38	58	49	26	3	1				16.1μ
H. siccans				1	1	4	7	22	39	66	41	15	4				16.8μ
H. gramineum	1			1	4	4	11	19	21	34	41	38	14	7	4	1	17.4μ
H. avenae					1	2	10	23	44	57	50	12	1				16.7μ

fungus, *Helminthosporium gramineum*, such parasites as *H. dictyoides* Drechsl., the cause of a fairly serious net blotch of meadow fescue, *Festuca elatior* L., and *H. siccans* Drechsl., the cause of a widespread leaf spot affecting perennial rye grass, *Lolium perenne* L., and Italian rye grass, *L. multiflorum* Lam. Like the bent grass parasite, *H. dictyoides* and *H. siccans* as well as *H. gramineum* produce conidial apparatus during June and the earlier weeks of July. Meadow fescue and the rye grasses are often found in fields intermingled with redtop, so that circumstances of local propinquity are present to encourage a supposition that the parasites on the grasses might belong to a single species. Yet, on closer examination, differences in conidial morphology become evident entailing separation of the parasites into species along lines corresponding to the generic separation of the host plants.

These differences are brought into relief in tables 1, 2 and 3, in which are shown the distribution in appropriate classes and the computed averages of measurements of the lengths and the diameters of 200 conidia taken from field material of the several members of the Pyrenophora series of most immediate concern, together with the corresponding distribution and averages of the numbers of septa in the conidia measured. The two separate sets of measurements of the bent-grass parasite are given under the binomial *Helminthosporium erythrospilum*, one set (*a*) being derived from spores taken from infected leaves of redtop collected near La Fayette, Ind., Aug. 25, 1928, the other set (*b*) from conidia taken from leaves of seaside bent collected at Arlington Experiment Farm, Sept. 19, 1928. The measurements of *H. dictyoides* were made on spores scraped from diseased leaves of meadow fescue gathered at Arlington Experiment Farm, June 7, 1934; and affected foliage of perennial rye grass gathered also at Arlington Experiment Farm, June 7, 1934, was used in making the measurements of *H. siccans*. Barley affected with stripe, originating from Virginia early

TABLE 3.—*Numbers of septa in 200 conidia produced by several species of Helminthosporium on their respective grass hosts in nature, distributed in classes*

Fungus	1	2	3	4	5	6	7	8	9	10	11	Average septation
H. erythrospilum (*a*)		7	17	40	24	71	27	11	2	1		5.4
H. erythrospilum (*b*)		6	19	37	40	50	25	19	3	1		5.4
H. dictyoides	5	23	20	61	41	35	10	3	1		1	4.4
H. siccans		13	13	50	64	35	14	6	4	1		4.9
H. gramineum	1	24	42	66	40	21	6					4.0
H. avenae	2	4	9	72	42	54	11	3	2		1	4.9

in June, 1934, was used in making the measurements of *H. gramineum*. Though *H. avenae* is hardly to be considered among the smaller members of the series, measurements of that species are included for comparison; they were made on conidia taken from leaves of oats, *Avena sativa* L., severely affected with leaf stripe collected at Arlington Experiment Farm, June 26, 1934.

The data given in tables 1–3 show in a somewhat quantitative manner relations that are readily perceptible when material of the several species are examined side by side. Though the conidia of the bent-grass parasite are appreciably shorter than those of *H. gramineum* and of *H. dictyoides*, the inferiority in length, especially in comparison to the stripe fungus, is not at all pronounced. On the other hand, the inferiority in width of conidium relative to any of the other four species is striking and undoubtedly significant, since this dimension is much less given to wide variation than is the length. Again, although the conidia of the bent-grass parasite are shorter than those of the other species represented in table 1–3, they are, nevertheless, on the whole more abundantly septate. As a result, the conidial segments are not only narrower than those of the other species but also shorter, and that in a measure more than proportional to the smaller length of the conidium.

From the accounts of Nisikado (7, 8) it is evident that the conidia of *Helminthosporium arundinis* Lév., a parasite causing leaf spot of *Arundo donax* L., approach those of the bent-grass fungus more closely in length, width, and frequency of septation, than do the conidia of either *H. dictyoides*, *H. siccans*, *H. gramineum* or *H. avenae*. With respect to shape, however, the correspondence would seem not especially close. The end cells in the conidia of *H. arundinis* are described as comparatively narrow; and this condition entails, as is apparent in Nisikado's figures, a more pronounced tapering of the spore in its apical and basal portions than is typical of the bent-grass parasite, whose conidia are as a rule abruptly rounded off at both ends. Moreover, the protruding position of the hilum set forth among the distinctive features of *H. arundinis* offers a marked contrast to the inclusion of the homologous scar within the basal contour of the conidium in the parasite on redtop and seaside bent.

Cultural Characters of the Parasite

The isolation of *Helminthosporium erythrospilum* offers no unusual difficulties. When vegetatively vigorous leaves with newly established lesions are available, the invaded portions of tissue may be excised, and after surface sterilization and repeated washing in sterile water, planted on some suitable medium like maize-meal agar. From most plantings thus made, mycelium of the parasite will grow out, free from contamination with

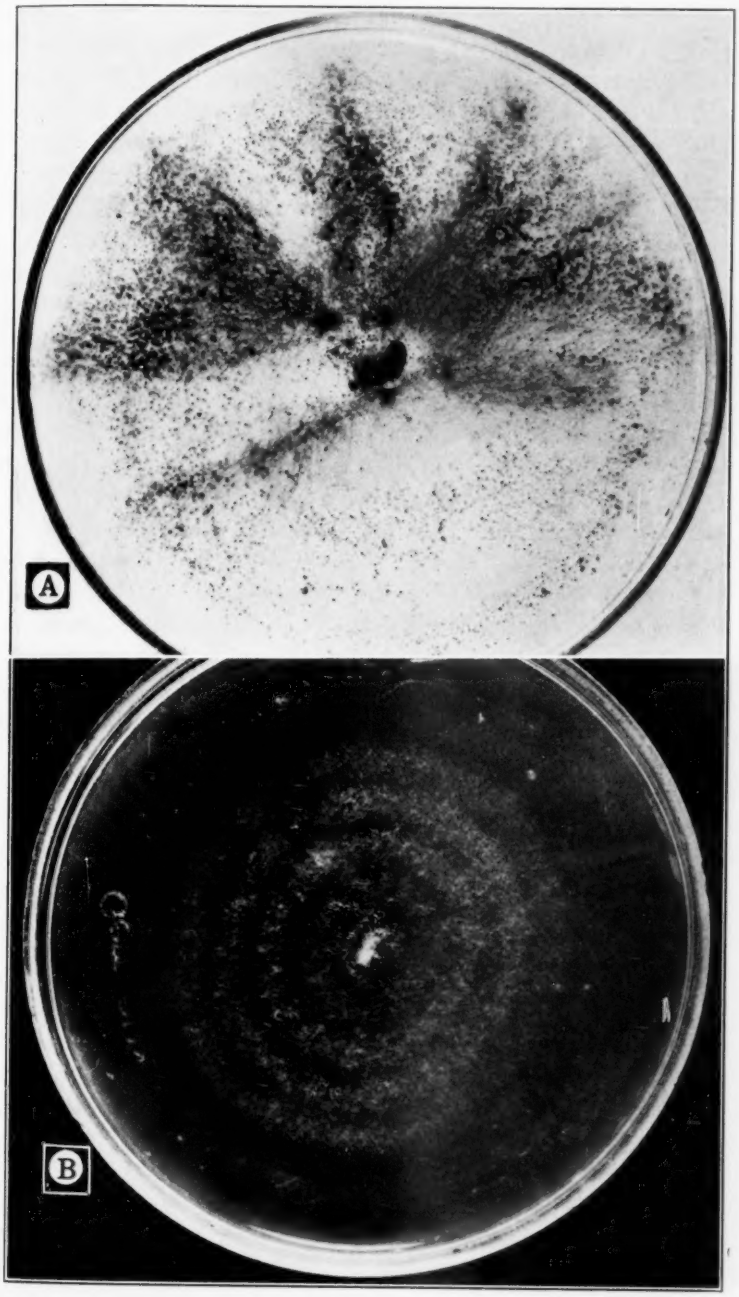

FIG. 4. Maizemeal-agar plate cultures of *Helminthosporium erythrospilum*. ×1. A. Culture incubated for 30 days at approximately 15° C., showing numerous submerged sclerotia. B. Culture after 6 days growth at a temperature of approximately 30° C., showing character of aerial mycelium.

other organisms. When withered leaves bearing conidial apparatus need to be used, plain agar dilution plates may advantageously be employed to make possible the removal of single germinating conidia to new cultures.

The fungus once isolated grows well on artificial media, showing a fairly rapid rate of linear extension. On maize-meal-agar plates at 30° C., it produces in several days a light grey or steel grey aerial mycelium (Fig. 4, B) rather strongly resembling that of *Helminthosporium siccans* (Fig. 5, B), though ordinarily somewhat less copious and often more given to zonation. Accordingly, like also the latter species, it is easily distinguished from *H. dictyoides,* which, in maize-meal-agar plate cultures, develops a more abundant, almost white, somewhat columnar or tufted mycelium (Fig. 5, A).

What is perhaps the most distinctive character of *Helminthosporium erythrospilum* on artificial media comes to light when cultures of it are kept at rather low temperatures (10 to 20° C.) for 2 weeks or longer. Numerous minute dark sclerotia scarcely exceeding 0.1 mm. in diameter make their appearance scattered through the substratum. A dense arrangement in the presence of abundant food materials (Fig. 4, A) yields to a more diffuse distribution in a less nourishing substratum (Fig. 6, B). Apparently, these sclerotia are of the same sort as the much larger but otherwise similar bodies that are produced in cultures of *H. bromi* and that evidently represent immature perithecia. Sclerotia much like those of *H. bromi,* and hence greatly exceeding those of the bent-grass parasite in size, are produced often on maize-meal-agar cultures of the Pyrenophora occurring fairly abundantly on old oat straw in Virginia, Maryland, and Delaware, during May and early June (Fig. 6, A).

The obvious disparity in size between the sclerotia of *Helminthosporium erythrospilum* and the sclerotia of the Pyrenophora on oats is of moment, especially as the two fungi, unlike most other members of the same natural series, sporulate fairly readily and fairly abundantly in pure culture. When, for example, maize-meal-agar plate cultures of the bent-grass parasite are kept for a period of 50 days at a temperature of approximately 18° C. and protected from evaporation, conidiophores (Fig. 7, A–F) abundantly laden with conidia (Fig. 7, A) are produced. In coloration, septation, and dimensions these conidia (Fig. 7, G, a–n) show little dissimilarity from those produced under natural conditions. Nor are the conidiophores essentially different from those produced in nature, though as might be expected from analogy with species of Helminthosporium referable to the Cochliobolus series, which in general sporulate very freely on artificial substrata, the prolonged conditions suitable for development permit these structures to attain greater length and to bear a proportionately larger number of conidia.

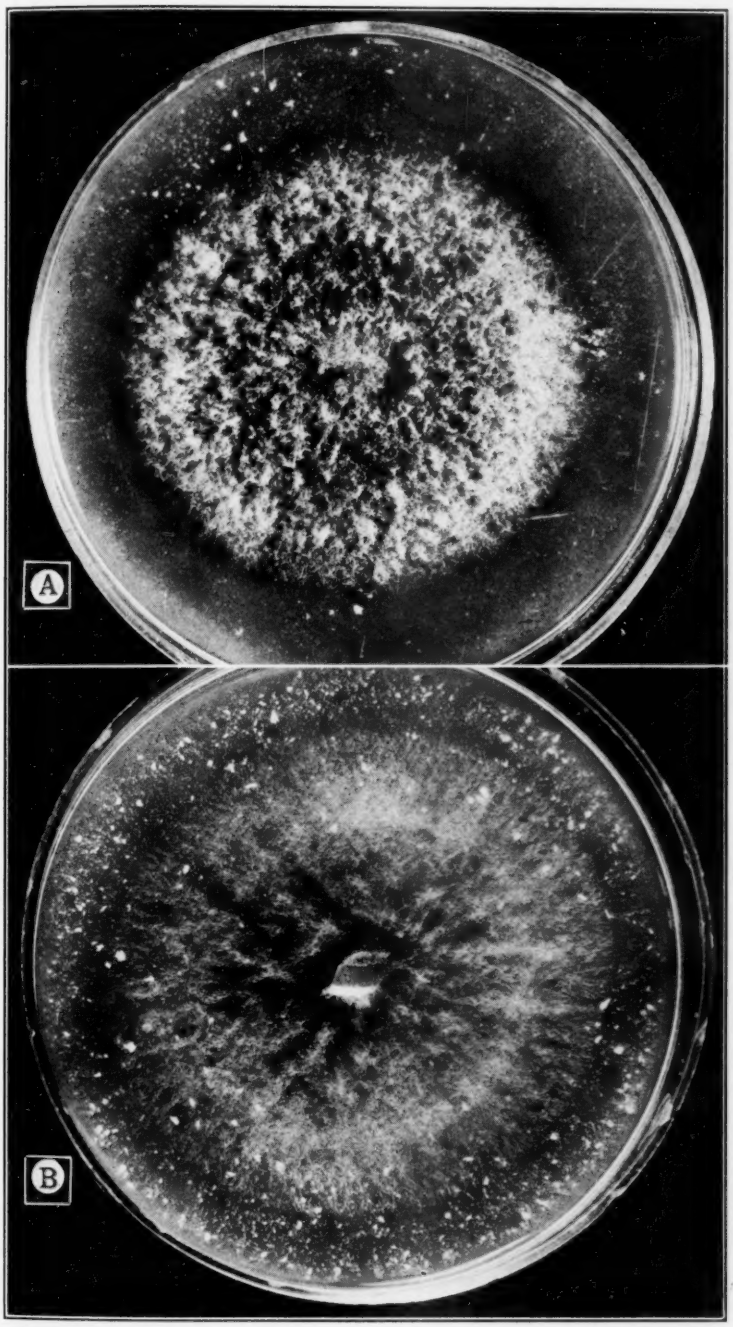

FIG. 5. Maizemeal-agar plate cultures of 2 species of Helminthosporium comparable to *H. erythrospilum* in conidial dimensions, showing appearance of aerial mycelia after 6 days' growth at a temperature of 30° C. ×1. A. *H. dictyoides*. B. *H. siccans*.

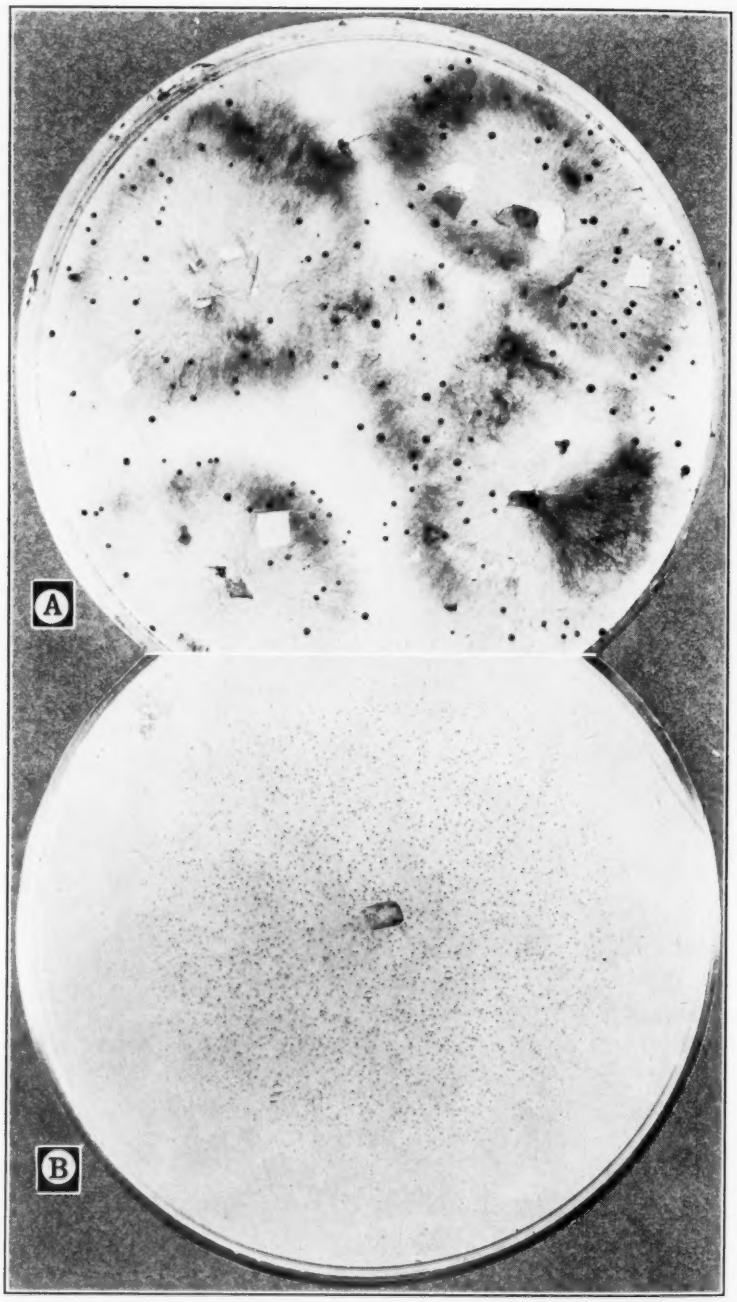

FIG. 6. A. Maizemeal-agar plate culture of the Pyrenophora sp. with ascospores having 5 transverse septa, that occurs abundantly on overwintered oat straw in Virginia, Maryland and Delaware; showing the relatively large sclerotia characteristic of the fungus. ×1. B. Maizemeal-decoction-agar plate culture of *Helminthosporium erythrospilum* after incubation for 30 days at a temperature of about 15° C.; the medium being poor in nutrients compared with that used in the culture shown in Fig. 4 A, has permitted the development of correspondingly fewer sclerotia. ×1.

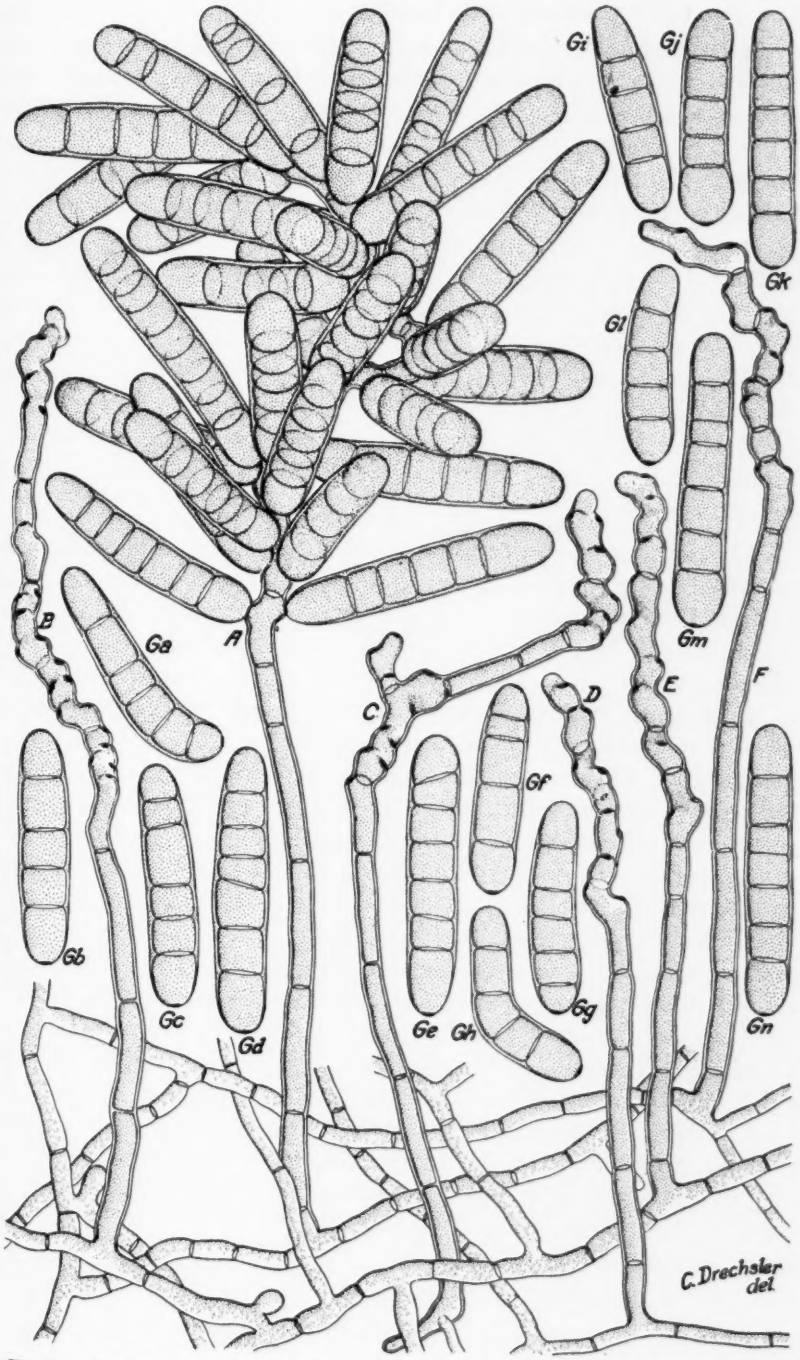

FIG. 7. *Helminthosporium erythospilum* in pure culture on a maizemeal-agar plate culture after incubation for 50 days at a temperature of approximately 18° C. ×500. A. Conidiophore with attached conidia. B–F. Denuded conidiophores. G, a–n. Conidia.

DESCRIPTION OF THE PARASITE AS A NEW SPECIES

As the parasite under consideration differs morphologically from all of the several congeneric forms assignable to the same series, it deserves description as a new species. The specific name, meaning "red spot," proposed for it is intended to be broadly descriptive of the coloration often present in leaf areas occupied by it, without, however, implying that such coloration constitutes an invariable diagnostic character of the fungus.

Helminthosporium erythrospilum, n. sp.

Sparsum; hyphis fertilibus erectis, fuligineis, simplicibus vel rarius furcatis, nodulosis, hinc illinc geniculatis, 75–275 μ longis, 6–9 μ crassis, 1–10 septatis; conidiis flavidis vel dilute olivaceis, typice rectis sed interdum nonnihil curvatis, cylindraceis, utrimque abrupte rotundatis, 2–10 septatis, ad septa non constrictis vel minute constrictis, saepius 25–105 × 8–16 μ, e cellulis pluribus germinantibus, hilo in membrana incluso.

Habitat in foliis vivis vel languidis Agrostidis albae, Agrostidis palustris et Agrostidis tenuis in Amer. bor.

Conidiophores erect, brown or fuliginous, simple or occasionally somewhat branched, emerging singly or less often in pairs or larger groups mostly from between epidermal cells but sometimes from stomata, measuring mostly 75 to 275 μ in length by 6 to 9 μ in diameter, producing the first conidium usually 75 to 125 μ from the base and later conidia at variable successive intervals marked by geniculations; containing 1 to 10 septa that delimit segments mostly 15 to 35 μ long.

Conidia when mature usually distinctly yellowish, sometimes even light olivaceous; typically straight, rarely somewhat curved, mostly nearly cylindrical, the ends rather abruptly rounded off, containing 2 to 10 septa that are usually not marked by constrictions, but at times may be associated with perceptible constrictions; measuring usually 25 to 105 μ (average 65 μ) in length by 8 to 16 μ (average 12.3 μ) in diameter; individually including the nonprotruding hilum within the basal contour; germinating rather indiscriminately from any or all cells, but usually more abundantly from the thin-walled basal and apical cells than from the intermediate cells.

Attacking leaves of redtop, *Agrostis alba,* and seaside bent, *A. palustris,* on which it produces reddish or yellowish red or russet brown spots with bleached centers, the local discoloration later becoming more or less obliterated with the withering of the individual leaves from tip toward base. Causing also localized lesions on the leaves of colonial bent, *A. tenuis.* In Connecticut, New York, District of Columbia, Maryland, Virginia, Ohio, Indiana, Michigan and Wisconsin.

SUMMARY

A leaf spot has been found to occur widely on redtop in some Eastern and Middle-Western States. Under wet conditions it gives rise to localized

lesions, these being marked individually by russet discoloration surrounding a relatively small, paler, central region. Local discoloration is less evident in dry weather, when infected leaves often wither in a manner suggestive of drought injury. The same disease occurs in severe form also on seaside bent. Lesions unaccompanied by withering effects have been observed on colonial bent.

Comparison is made between the disease and a much less injurious leaf spot attributable to *Helminthosporium triseptatum.*

The disease is caused by a species of Helminthosporium producing conidia typically straight cylindrical, rounded abruptly at both ends, distinctly yellowish in coloration, 25 to 105 μ long by 8 to 16 μ wide, germinating by the production of germ-tubes from any or all segments. The fungus is, therefore, referable to the series having ascigerous connections in Pyrenophora. It is described as a new species, *H. erythrospilum,* being distinguished from various other members of that series—*H. gramineum, H. dictyoides, H. siccans* and *H. avenae*—by the smaller diameter and closer septation of its conidia; from *H. arundinis* by its conidia being abruptly rounded at both ends, and by the position of the hilum within the contour of the basal segment. In pure culture on artificial media it often gives rise to submerged sclerotia that are more numerous and much smaller than those produced under similar conditions by *H. bromi;* or than those produced in pure culture by the Pyrenophora having ascospores with typically 5 transverse septa that occurs abundantly on overwintered oat straw in the Middle Atlantic States.

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THE USE OF THE QUINHYDRONE AND ANTIMONY ELECTRODES FOR DETERMINING THE pH OF SOLID CULTURE MEDIA[1]

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In some studies conducted by the writer on the relation of the hydrogen-ion concentration of culture media to the growth of certain fungi, it became necessary to determine accurately the pH of solid culture media. The accuracy of colorimetric readings made on this type of media is so often questionable, due to the turbidity of the medium and the other errors to which this method is subject, that an effort was made to adapt the electrometric method.

The hydrogen electrode can not be used in solid media unless the buffer capacity of the particular medium is sufficient so that it can be diluted to the point where it will not solidify without altering the hydrogen-ion concentration. Lisse, Jensen, and Tittsler (8) found that Bacto-nutrient agar is sufficiently buffered to permit a 7-fold dilution without appreciably changing the pH. At this dilution, the medium remained liquid, and they were able to use the hydrogen electrode. The same authors report satisfactory results with the quinhydrone electrode in diluted Bacto-nutrient agar, and they prefer this electrode to the hydrogen electrode because it is much simpler to use.

Biilmann and Lund (5) and Biilmann (3) have shown that the quinhydrone electrode is sufficiently accurate to justify its replacing the hydrogen electrode in many instances. Later, Biilman, Klit, and Swaetichin (4) reported that the quinhydrone electrode gave correct pH values in phosphate mixtures up to pH 7.73; above this point the values were slightly too low, but they were fairly accurate even at pH 8.04.

The quinhydrone electrode was used by Watson (9) to follow the changes in pH in Swiss cheese during the process of making. The cheese and quinhydrone were mixed together in a mortar, packed in a glass tube, and then the electrode was inserted into the mixture.

Barnes and Simon (1) found that the antimony electrode could be used in determining the reaction of soils where the quinhydrone electrode could not be used because of the presence of considerable manganese. They worked out 3 equations to be used in changing the voltage obtained with the antimony electrode to pH units.

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station, Wooster, Ohio.

APPARATUS AND MATERIALS

A Leeds and Northrup Type-K potentiometer, Type-R galvanometer, Eppley standard cell, and storage batteries constituted the equipment for measuring the voltages. The Leeds and Northrup quinhydrone pH indicator No. 7654 was substituted for the apparatus mentioned above in some of the later determinations and proved to be quite satisfactory. The quinhydrone electrodes were made by fusing about 4 cm. of 21-guage platinum wire into the end of a piece of glass tubing. The protruding end of the wire was shaped into a coil. A saturated calomel half cell served as a reference electrode and a 2 per cent agar bridge, saturated with KCl served as a junction.

The antimony electrode was of the type described by Barnes and Simon (1) and consisted of an antimony rod cast from C.P. antimony soldered at one end to a copper wire. De Khotinski cement was used to seal the electrode into a glass tube so that about one inch of the antimony rod projected from one end of the glass tube and the copper wire extended through the opposite end.

EXPERIMENTAL

Bacto-nutrient, Bacto-corn-meal, Bacto-Lima bean, Bacto-bean pod, and Bacto-prune agars were made up from the dehydrated preparations of the Difco Laboratories, Detroit, Michigan, according to the manufacturer's recommendations. Potato-dextrose agar was included and was prepared according to the usual laboratory procedure. After autoclaving, a plate was poured from each lot of media, and then portions of each medium were diluted with distilled water 3, 5, 7 and 10 times and the diluted portions poured into Petri plates. Prune agar was the only one that solidified when diluted 5 times. All the others were semi-liquid at this dilution and all were liquid, or extremely soft, at dilutions of 7 and 10 times. The pH of the distilled water used was $5.9 \pm .2$.

The hydrogen-ion concentration measurements were made on the liquid samples by mixing quinhydrone with a small portion of the medium in a vial and then using the quinhydrone electrode. The reading was taken as soon after adding quinhydrone as possible. Readings were obtained on the samples that solidified by using the following procedure: A small amount of quinhydrone was sifted on an area, about 1 inch in diameter, of the surface of the medium. Then the quinhydrone and agar were mixed together by using a spatula. The electrode and free end of the KCl-agar bridge were placed immediately in the mixture of agar and quinhydrone, and the voltage reading taken.

Voltages were converted to pH by use of the standard conversion tables furnished by Leeds and Northrup. The laboratory temperature during

all of the work was approximately 25° C. The pH determinations on the various dilutions of 2 lots of the different media prepared at 2 different times are given in table 1.

TABLE 1.—*Effect of dilution on the pH of solid culture media, determined by the quinhydrone electrode*

Medium	Dilution	pH		Medium	Dilution	pH	
		Lot 1	Lot 2			Lot 1	Lot 2
Nutrient	None	7.33	7.32	Prune	None	6.27	6.16
	1:3	7.31	7.16		1:3	6.23	6.07
	1:5	7.21	7.06		1:5	6.20	6.04
	1:7	7.09	6.99		1:7	6.18	6.04
	1:10	6.96	6.92		1:10	6.21	6.02
Bean-pod	None	5.72	5.73	Lima bean	None	6.16	6.25
	1:3	5.85	5.77		1:3	6.32	6.28
	1:5	5.85	5.77		1:5	6.33	6.28
	1:7	5.94	5.77		1:7	6.36	6.27
	1:10	5.94	5.74		1:10	6.42	6.34
Corn meal	None	6.06	5.82	Potato	None	5.67	5.64
	1:3	6.16	5.85	dextrose	1:3	5.80	5.85
	1:5	6.18	5.97		1:5	5.86	5.88
	1:7	6.33	5.99		1:7	5.91	5.91
	1:10	6.67	6.24		1:10	5.94	5.95

Potato-dextrose agar was adjusted by the addition of H_3PO_4, mono-, di-, and tri-sodium phosphates after sterilization and before solidification so that a series of agars ranging from a very acid reaction to well above neutrality was obtained. The pH of each was determined colorimetrically by using a comparator and following the methods of Clark and Lubs (6). A small sample of each agar was mixed with quinhydrone in a mortar and the pH determined with the quinhydrone electrode as indicated above. Also, the quinhydrone was mixed with the agar, which had solidified in a Petri dish, by lowering and raising the electrode and bridge a few times into and out of the agar after the quinhydrone had been sifted over a small area of the surface of the medium. The pH was also determined by using an antimony electrode. This electrode and the bridge were merely inserted into the agar in a Petri dish; the voltage was read as soon as equilibrium was reached; and then the voltage was converted to pH by the use of the equations given by Barnes and Simon (1). Results obtained by the 4 different methods on 2 series of agars prepared at different times are given in table 2.

TABLE 2.—*Comparison of the quinhydrone and the antimony electrodes with the colorimetric method for determining the pH of solid media*

Agar number	pH by colorimetric method		pH by mixing quinhydrone with agar in a mortar		pH by sifting quinhydrone on surface of agar		pH by antimony electrode	
	Series 1	*Series 2*	*Series 1*	*Series 2*	*Series 1*	*Series 2*	*Series 1*	*Series 2*
1	2.0	2.2	2.00	2.13	2.11	2.15	2.24	2.20
2	2.6	3.2	2.60	3.18	2.64	3.19	2.84	3.11
3	2.8	4.4	2.89	4.39	2.93	4.39	3.09	4.82
4	3.8	6.0	3.83	5.86	3.82	5.93	4.16	5.95
5	5.2	6.4	5.48	6.28	5.48	6.28	5.78	6.58
6	6.0	6.6	6.18	6.54	6.18	6.56	6.34	6.66
7	7.0	7.0	6.83	6.90	6.84	6.92	6.99	7.15
8	7.4	7.2	7.15	7.00	7.16	7.07	7.26	7.12
9	7.8	7.4	7.45	7.10	7.45	7.13	7.56	7.31
10		7.6		7.26		7.28		7.39

DISCUSSION

The results given in table 1 show that most of the common culture media used by plant pathologists are not buffered sufficiently so that they can be diluted without appreciably changing the pH. In most cases the pH of the media used was altered by 0.1 to 0.2 of a pH integer when diluted 5 times. Kolthoff (7) has studied the effect of dilution on the pH of buffer mixtures and found that a dilution of 2 times changes the pH of acetic acid-sodium acetate, potassium biphthalate-sodium hydroxide, mono-potassium phosphate-sodium hydroxide, and mono- and di-potassium citrate buffer mixtures a detectable amount. Diluting these buffers 5 times altered the pH almost as much as a similar dilution changed the reaction of the media listed in table 1. An accurate pH determination on many solid media evidently can not be expected or obtained by making a reading on a sample of the medium diluted to the extent that it remains liquid.

Determinations made with the quinhydrone electrode on solid media compare well with colorimetric readings up to neutrality, table 2. Above this point, however, the quinhydrone readings are slightly too low. Agars reading pH 7.2, 7.4, and 7.8 colorimetrically gave readings of 7.07, 7.16, and 7.45, respectively, with the quinhydrone electrode. If the colorimetric readings are accurate, and they probably are more nearly so than determinations made with the hydrogen electrode on diluted media, the quinhydrone electrode values are 0.13, 0.24 and 0.35 pH units too low. Lisse, Jensen, and Tittsler (8) reported that the quinhydrone electrode gave values 0.16 pH lower than those obtained by the hydrogen electrode in diluted nutrient agar of which the pH, as determined by the hydrogen

electrode, was 7.2 to 7.3. Evidently the quinhydrone electrode does not give accurate readings at as high pH values in agar media as it does in buffered solutions, since Biilmann, Klit, and Swaetichin (4) found that correct values were obtained in phosphate mixtures up to pH 7.73, and above this point the values were slightly too low but they were fair even at pH 8.04.

The values obtained with the antimony electrode do not check as closely with the colorimetric readings as those obtained with the quinhydrone electrode. Above neutrality the antimony electrode also gave values which were too low. More trouble was experienced in making readings with this electrode than with the quinhydrone; mainly because it was slower in coming to equilibrium. In general, it was less satisfactory than the quinhydrone electrode.

The electrometric method of measuring the pH of solid culture media that are acid or only slightly alkaline by use of the quinhydrone electrode has several advantages over the colorimetric method. An accurate reading can be quickly made without melting the agar and without any of the difficulties experienced with the colorimetric method due to the turbidity of the media. The electrometric apparatus requires much less attention than the buffer mixtures and standards for colorimetric determinations. Readings can be made on small amounts of medium. For instance, the hydrogen-ion concentration of an agar medium under an organism can be measured by inverting the layer of medium in a culture dish and making the determination on that part of the medium below the organism.

A somewhat serious criticism of the quinhydrone electrode is that the potential is likely to drift. Because of this characteristic it is necessary to make the readings at some definite time. Lisse, Jensen, and Tittsler (8), in an effort to eliminate the effect of the drift, extrapolated the values to zero time. The data given by these authors show that in nutrient agar the pH readings obtained after 1 minute are, on the average, about 0.03 of a unit lower than when they are extrapolated to zero time. Baver (2) found that the first reading made not later than 1 minute after immersion of the electrode was the best one. For most work the first reading, taken not later that 1 minute after immersion, should be sufficiently accurate.

If the reaction of the medium is above pH 7.1, the readings obtained with the quinhydrone electrode will be too low and corrections should be made.

SUMMARY

The effect of dilution on the pH of the following agar media has been studied: Nutrient, corn-meal, Lima bean, bean-pod, prune, and potato-dextrose. Diluting these media with distilled water 5 times, at which dilution all except prune agar were liquid or semi-liquid, altered the pH by 0.1 to

0.2 of a unit as determined by the quinhydrone electrode. An accurate pH determination can not be made on these agar media by diluting them to the point where they remain liquid.

The quinhydrone electrode gave pH readings that compared well with colorimetric readings on solid potato-dextrose agar media from pH 2.0 to 7.1. Above pH 7.1, readings obtained with this electrode were too low. Determinations made with this electrode checked more closely with colorimetric readings than did those obtained with an antimony electrode. The antimony electrode also gave readings that were too low when used in alkaline media.

The quinhydrone electrode furnishes a simple, quick, and accurate electrometric method of determining the pH of acid or slightly alkaline solid culture media. It is much more satisfactory than the colorimetric method over the pH range where it can be used.

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THE USE OF OIL-SOLUBLE COPPER AS A FUNGICIDE

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Copper, as used in the spraying of plants, has been applied almost entirely in standard methods such as Bordeaux or other forms of water-carried copper, the copper being either in a suspension form, as in Bordeaux, or the ammonical-copper solution in water. In the former type the copper is present in a gelatinous form, in the latter as a solution, but restricted in its penetration by reason of the water carrier. Such applications must of necessity deposit the copper on the surface of the leaf and twig with but little possibility of absorption within the tissue of the plant itself. Copper absorption into the leaf from Bordeaux sprays has been noted,[2] but only in minute quantities and particularly through injured cuticle. The literature as a whole indicates surface deposits only[3] rather than penetration into the cellular structure of the plant where the mycelium of most fungi is found. It is generally recognized that, once the germ tube of a spore penetrates into tissue, it then becomes difficult to kill. This difficulty is due primarily to the use of surface deposits of the fungicides commonly selected. To overcome this difficulty it is necessary that we should have fungicides capable of penetrating plant tissue or solvents for fungicides that will penetrate into the tissue of the plant. The principle is similar to the search for chemicals that will kill the bacteria of pear blight within the twig.

The use of oil-soluble copper compounds has been found to accomplish much of this desired purpose and to overcome the criticism of surface deposit of metallic fungicides. It has been shown that most oils, even oil emulsions, penetrate readily[4] through stomata and even through epidermal cells and in this way offer possibilities of carrying oil-soluble toxics into the tissue. To accomplish this purpose, of course, it is necessary to have solutions of copper rather than copper suspensions, as the latter would usually if not in all cases be sieved out by the cell walls. The following experiments indicate that the copper is carried into the plant wherever the oil penetrates and that in this way it is possible to carry fungicides into

[1] Consulting Entomologist, San Francisco, California.

[2] Barker, B. T. P., and C. T. Gimingham. Further Observations on the Fungicidal Action of Bordeaux Mixtures. Jour. Agr. Sci. VI: Part II, 1914.

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[4] de Ong, E. R., Knight, Hugh, and Chemberlin, J. C. A Preliminary Study of Petroleum Oil as an Insecticide for Citrus Trees. Univ. of California Hilgardia 2, No. 9, 1927.

plant tissue. The organic form of copper known as copper resinate, $Cu(C_{20}H_{29}O_2)2$, is dissolved in a pine-tar oil and the combination made water soluble and diluted as desired. The oil used has been developed especially from the standpoint of plant tolerance and is known to the trade as "Palustrex." It is especially desirable to use oils of a high plant tolerance, for such work as oils that oxidize readily or that naturally carry large amounts of organic acids are too dangerous for use on plants. Copper resinate dissolves readily in this form of pine tar oil and may be blended with petroleum or used in water-soluble form either alone or with petroleum and may also, if desired, carry nicotine where a combination insecticide and fungicide is necessary. Such combinations of copper resinate and Palustrex, as mentioned above, have been in common use in California for the last 3 years, in field work, with increasing popularity and with decidedly less metallic copper than is required to secure similar control with Bordeaux. The inference was that the more perfect distribution both on the surface and within the tissue of the plant of the copper applied gave better control than that applied as a surface application and subject to loss by weathering. To confirm this theory, the following experiments were made:

A heavy Bordeaux spray was prepared to check against the copper-oil spray, each concentrate containing 1.33 per cent of metallic copper. These are, of course, excessive amounts over that commonly used for spray purposes but, since the colorimetric method of determining copper does not detect very minute quantities, it was found necessary to use the undiluted copper-oil spray and to make a correspondingly heavy Bordeaux in order to have for determinations sufficient copper present after given intervals of time. The trial was made both on apricot leaves and twigs and plum twigs in the late fall but before the leaves had fallen. After 30 days, determinations of copper were made both on the surface and on the interior of the twig with the following results: The Bordeaux showed 80 per cent recovery from the surface of the twigs but no copper could be found in the tissue by digesting the twigs after the removal of the surface copper. The oil-coated twigs were still quite oily at the end of the 30-day period because of the heavy application made, and approximately 60 per cent of the copper was recovered from the surface of the twigs; but then, after thorough cleansing in a weak nitric acid solution, a determination of the copper present in the tissue itself showed 21 per cent of the total amount applied, leaving 19 per cent unaccounted for by weathering. It should be noted, however, that the colorimetric method used gives only approximate readings. These findings confirm the theory that oil may be carried into the tissue of the leaf and twig and to such a degree as to be recovered in demonstrable amounts.

The method used in determining the copper present follows that outlined in Bulletin No. 785, U. S. D. A., from which the following abstracts are made:

The reagents used: copper sulfate crystals, 3.928 grams dissolved in 1,000 cc. of distilled water; potassium ferrocyanid, 2 grams dissolved in 100 cc. of distilled water.

Stock dilutions of the copper sulfate solution are prepared each containing one-half cc., 1 cc., 1½ cc., 2 cc., and so on up to 10 cc., in 100 cc. of distilled water. These dilutions would then contain from one-half to 10 milligrams of copper per 100 cc.

Leaves or twigs for testing are collected usually in amounts of 200 gram quantities, each washed separately in 1,000 cc. of distilled water containing 2 cc. of c.p. nitric acid. Colormetric standards are made up of 5 cc. portions of the graded stock solution of copper sulfate containing a few drops of potassium ferrocyanid solution to give typical color reactions. Similar portions of washings of leaf and twig samples may then be prepared with the indicator added. Match the samples of standard copper solutions against those of the washings showing similar color; multiplying this reading by 5 gives a value in milligrams of copper per 100 grams of fresh leaf sample.

To determine copper within the tissue, a digestion method[5] was applied similar to that used in arsenical determinations where apple parings are digested in nitric acid following the addition of sulfuric acid and evaporated down to almost dryness. The residue is dissolved again in a weak nitric acid solution and determinations made according to the previous procedure.

[5] Martin, Hubert. Studies upon the Copper Fungicides. II. Some modifications of Bordeaux Mixture designed to overcome practical difficulties in its applications. Annals of Applied Biology, XX, 2: 342–363, 1933.

A BACTERIOPHAGE IN RELATION TO STEWART'S DISEASE OF CORN[1]

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A form of transmissible lysis or bacteriophage has been found in some instances associated with *Aplanobacter stewarti* (E. F. Smith) McCulloch, the organism that causes Stewart's disease of corn. The most favorable material for the isolation of a bacteriophage has been the roots and lower nodes of corn plants killed by the disease, grain from a badly infected crop, and the lower nodes and pith of corn plants that for a time showed the leaf symptoms of Stewart's disease and that later recovered.

The first test for the presence of a phage was made in November, 1933. The basal portion of corn plants that had wilted and dried in midsummer of that year were used, the material minced, being placed in a flask, covered with nutrient broth, and then set aside at room temperature for 48 hours. The liquid was next passed several times through filter paper, and finally, through a Chamberland-Pasteur L 3 cylinder, using suction equivalent to about 10 cm. of mercury. To tubes containing 10 cc. of Bacto-nutrient broth, pH 6.8, seeded with 5 drops of a 24-hour-old culture of *Aplanobacter stewarti*, the filtrate was added in graded quantities of 7, 14, and 20 drops. In each set of 5 tubes 1 was reserved for a culture check and 1 for testing sterility of filtrate.

After incubation for 24 hours at 25° C., inhibition was evident in tubes to which the filtrate had been added. Serial passages gradually increased the titer. As the activity of the phage was enhanced, manifested by more complete inhibition or lysis, the amount of filtrate to new cultures was gradually reduced to 3, 5, and 7 drops, regardless of the quantity of medium used. At the 38th passage inhibition was demonstrated at a dilution of 10^{-7}. Even at the point of greatest activity of the phage filtrate, the bacterial cultures were not sterilized by the phage; yet the character of the growth was profoundly changed. No pellicle developed, the broth remained clear for 3 or 5 days, and later a heavy, coarse sediment formed, accompanied by slight clouding of the liquid. All isolations of *Aplanobacter stewarti* were not equally affected by the phage.

Physiological changes in the corn-wilt organism induced by the phage were further demonstrated by the intimate association of bacterial culture and phage filtrate upon potato-dextrose agar. These changes were evidenced by variation or loss of yellow color, increase or decrease of viscosity of bacterial growth, and by reduction or loss of virulence. The titer or state of activity of the phage was found to be an important factor. As the titer increased, inhibition upon solid media became more definite, until

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station.

finally very little growth occurred. These modifications of bacterial growth, thus artificially obtained, seemed to duplicate the variants or strains of *Aplanobacter stewarti* that were frequently isolated from diseased corn plants. After a mixed culture of bacteria and phage had been grown upon solid medium for 6 generations, the phage was readily reclaimed.

A quantity of seed corn, collected from a badly infected crop in September, 1933, was shelled and stored in the laboratory at room temperature. No difficulty was experienced at any time in isolating from this grain a phage that manifested inhibition for cultures of the wilt organism obtained from diseased plants of the same crop as the seed. This seed, when planted, was found to develop only 5 per cent of infected plants.

It was then decided to test the effect upon naturally infected grain of treatment with a bacteriophage of relatively high titer. Cultures were prepared in quantity, using 500 cc. units of nutrient broth inoculated with 5 drops of a 24-hour-old growth of *Aplanobacter stewarti*. As soon as faint clouding was noticeable, 5 drops of the phage filtrate were added to each culture. After 48 hours' incubation, the lysed cultures were filtered through a Berkefeld cylinder and the liquid used for seed treatment. The grain was soaked in the filtrate first for 24 hours in an ice-box at 50° F. and then for the same period at room temperature. After draining and partially drying in air, the corn was planted.

Eighteen lots of commercial seed, obtained from different seed firms, were used in these tests. Plots were located upon a great variety of soils. The average amount of infection where phage-treated seed was used was 1.4 per cent, whereas the average infection for the nontreated check plots was 18 per cent. Insects, including chinch bugs, 12-spot cucumber beetles, and flea-beetles, were present in all plots. There appeared to be less spread of the disease in the plantings from seed treated with the phage than in the check plots. In the treated plots the amount of infection ranged from none to 4 per cent, whereas untreated plots varied from none to over 40 per cent.

Examinations have been made of corn plants that exhibited mild infections or that appeared to be in a stage of recovery. In many cases the organisms isolated were found to have a phage associated with them. When mixed cultures of bacteria and phage were injected into healthy plants, in some instances typical leaf striping occurred, followed by recovery; in others, no symptoms developed.

The facts presented suggest that a bacteriophage may play an important rôle in reducing losses due to Stewart's disease. The presence of the phage in plants recovering from the disease and in seed that, although taken from diseased stock, produced relatively few diseased plants lends support to the idea. The most important evidence, however, is gained from the results of seed treatment, by which the disease was reduced in every case to a minimum.

PHYTOPATHOLOGICAL NOTES

Rate of Increase in Area of Apple-Spur Leaves.[1]—The period between the delayed dormant and the calyx- or petal-fall stage is generally accepted as the critical period for apple scab (*Venturia inaequalis* (Cke.) Aderh.) infection and control. It is during this period that the areas of the apple-spur leaves are undergoing their most rapid development. Since the unfolding and increase in area of apple leaves has an effect on the subsequent distribution of spray material on the leaf surface, which may affect the degree of protection afforded, we determined to measure the area of apple-spur leaves at 2 and 3 day intervals during this critical period.

One hundred approximately uniform flower spurs, each generally bearing 11 leaves, which is characteristic of the variety, were selected for each determination from 3 Rome Beauty apple trees from April 25 to May 10. Each sample of 100 spurs was divided into several equal lots and the area of the leaves of each lot determined after removing them from the spurs and placing them in the measuring apparatus. In this manner it was possible to observe the variations in leaf area that were present. The areas were

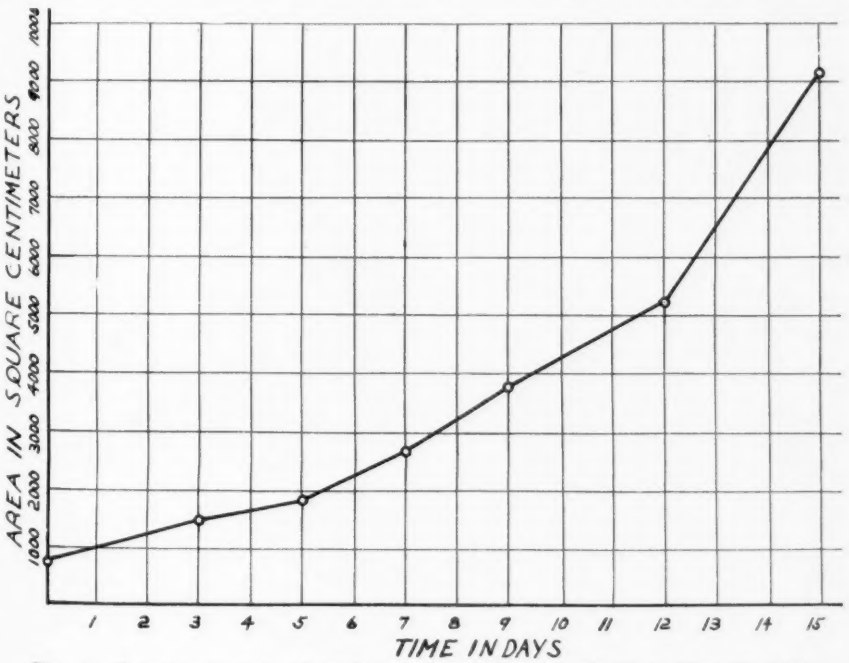

FIG. 1. Increase in area of apple-spur leaves from April 25 to May 10, 1934.

[1] The writers wish to express their sincere appreciation to C. L. Burkholder of the Department of Horticulture, Purdue University Agricultural Experiment Station, for cooperation in this work.

measured by the photoelectric device described by Withrow,[2] which is accurate to approximately 1 per cent. The greatest variation in area between individual lots of the same sample was approximately 10 per cent. Since a variation in the leaf area between spurs occurs, care in sampling and the use of a large number of spurs is necessary in a determination of this kind.

In figure 1 the area in square centimeters of 100 spurs is plotted against time in days. The rate of increase in total area of the leaves from April 25 to May 10 became progressively greater. During the period of 15 days the total leaf area increased to 9 times the original value. The growth of flowering apple spurs does not progress at the same rate in all seasons. For example, in 1933, the rate of growth was slower than in 1934, and 21 days were required for the passage of fruit spurs through similar stages of development. Fruit spurs and leaves representative of the samples taken April 25, May 2, and May 10, 1934, are shown in figure 2.

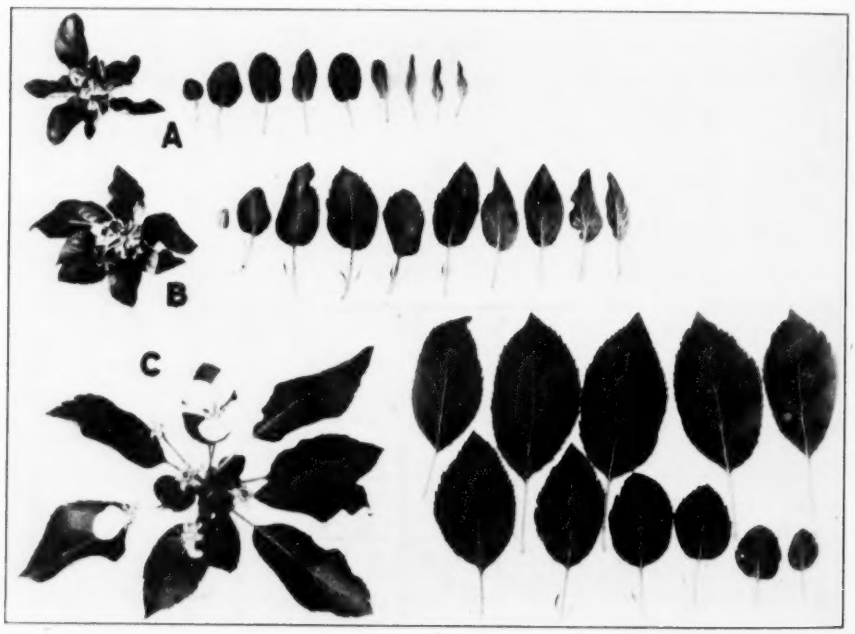

FIG. 2. Rome apple spurs and leaves representative of the samples taken on April 24, May 2, and May 10, 1934. A. Spur in the prepink stage, having only the first 5 leaves on the spur unfolded. B. Pink stage, 7 days later, when the last leaf has unfolded and may be covered with spray. C. Rome spur in the early petal-fall stage taken 15 days later than A. $\times 0.2$.

[2] Withrow, R. B. A photoelectric device for the rapid measurement of leaf area. Unpublished. Manuscript on file in the Department of Horticulture, Purdue University, La Fayette, Indiana.

The rapid rate of unfolding and increase in area of the young leaves, particularly prior to the pink-bud stage, emphasizes one of the difficulties encountered in practice in protecting the foliage from infection with sprays applied during this early period.—R. C. BAINES and H. M. BENEDICT, Purdue University Agricultural Experiment Station, La Fayette, Indiana.

Hybridization Between Sphacelotheca sorghi and Sorosporium reilianum.—Recently the writers succeeded in crossing the covered kernel smut of sorghum, *Sphacelotheca sorghi* (Link) Clinton, and the head smut of

FIG. 1. A head of Waconia orange sorgo infected with hybrid smut produced by injecting hypodermically into the young plant a monosporidial culture of *Sphacelotheca sorghi* combined with a monosporidial culture of *Sorosporium reilianum*. Note the horn-like sori and floral proliferation.

sorghum, *Sorosporium reilianum* (Kühn) McAlpine. Two monosporidial lines of *Sphacelotheca sorghi* and four of *Sorosporium reilianum* were grown singly in flasks of potato-dextrose broth, after which sorghum plants were inoculated by means of a hypodermic syringe with the broth cultures of single lines and with paired combinations. None of the single lines caused infection, but, after an incubation period of from 7 to 10 days, chlorotic flecking such as commonly appears on sorghum plants injected with combinations of sexually compatible monosporidial lines of *Sphacelotheca sorghi* or of *Sorosporium reilianum* appeared on 2 lots of plants which had been injected with different combinations between monosporidial lines of the 2 species. When the plants approached maturity, sori containing chlamydospores were developed in the inflorescences of some of the plants that had previously shown chlorotic flecking.

Sori and spores both appeared to be somewhat intermediate in type between those of *Sphacelotheca sorghi* and *Sorosporium reilianum*. The sorus tissue developed chiefly from the ovaries and closely associated floral parts, as is the case with *Sphacelotheca sorghi,* but sori were much longer and more irregular in shape than those of this smut. The leathery white peridium covering the sori and the abundant floral proliferation resemble those of *Sorosporium reilianum* (Fig. 1). Chlamydospores were somewhat larger than those of *Sphacelotheca sorghi* but within the range for those of *Sorosporium reilianum*. The walls were finely echinulate, much less distinctly so than those of *Sorosporium reilianum*, but not smooth like those of *Sphacelotheca sorghi*. In this respect the new smut, therefore, is also intermediate in type. The chlamydospores germinate abundantly, producing very long, slender promycelia somewhat like those of *Sphacelotheca sorghi*. Sporidia are produced sparsely and resemble those of *Sphacelotheca sorghi,* being more slender and tapering than is typical for those of *Sorosporium reilianum*. Frequently, some promycelia produce numerous hyphal branches with very few or no sporidia.

Studies on cultural characters, pathogenicity, and genetics of the new hybrid are now under way and will be reported later.—LEON J. TYLER and CLYDE P. SHUMWAY, University Farm, St. Paul, Minn.